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CHARACTERISTICS OF FOREST SOILS OF THE NORTHWESTERN UNITED STATES

W. L. POWERS

Oregon Agricultural Experiment Station[1]

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A large part of the farm land in the northern humid, temperate climates was developed under forest cover. This is true of much of the land now under cultivation in the Northwest. A large part of the Northwestern States is most suitable for forest production permanently. Approximately one-third of Oregon is commercial forest and in the Northwest there occur some of the finest forest soils in the world.

A recent investigation by the author, of organic soil colloids separated from peaty soil profile layers, indicates unusually high base exchange capacity in mixtures of organic and inorganic soil colloid. In forest soils the organic litter, fermenting layer, humified layer, and soil humus layer of different forest types should afford favorable material for studying the trend of humification, reaction, base capacity, and related characteristics. Here every degree of decomposition and also a range of natural mixtures of organic and inorganic constituents can be obtained. The cycle of transformations of organic constituents is now recognized as an outstanding factor in forest soil fertility.

Very little exact data are available on forest soils, particularly of the Northwest, or the effects of forest organic matter on soil fertility, yet foresters recognize the fundamental rôle of the organic matter in forest soil fertility. Information is also needed as to the best methods of improvement of newly cleared logged-off land where location, soil, and topographic conditions are attractive for crop production. An investigation has therefore been undertaken to study the significance of base exchange and other characteristics of typical Northwestern soil profile layers, and results thus far obtained are reported herein.

It is interesting to note that Gedroiz in 1922 recognized the fact that humic bases as well as zeolitic bases are concerned with base absorption. Since he was not able to separate these he assumed that the humic portion behaves similarly to the zeolitic. Recently Albin (1), McGeorge (5), and the writer (9) have shown that peat soils possess high base exchange capacity; in the case of organic soil colloids this has been found to be especially great and appears to reside in the lignin fraction (9). Decomposition results in a higher concentration of lignin and a consequent increase in base exchange capacity.

[1] Published as technical paper No. 176, with approval of director, Oregon Agricultural Experiment Station. Contribution from department of soils.

EXPERIMENTAL

The plan followed in this study has been to collect profile layer samples from typical forested areas for use in laboratory determinations. Nine soil profiles were collected, including three from the coast region, three from the Willamette water shed, and three from eastern Oregon, representing redwood (*Sequoia sonpervirous* [Lamb] Anal.), yellow pine (*Pinus ponderosa*, Douglas), Douglas fir (*Pseudotagua taxifolia* [Lamb] Britt), and oak (*Querous Carryana*, Douglas) land. Samples were taken of the litter, fermenting layer, fermented layer, soil-humus layer, the leached eluvial inorganic soil layer, and the illuvial or B horizon, which is the zone of accumulation. In some cases a sample of underlying soil material was obtained. Podzolic, lateritic, and brownerth soil types are included.

Determinations made and methods used thus far include (*a*) colorimetric determination of reaction value, or pH; (*b*) adsorption of moisture vapor over 3.3 per cent sulfuric acid; (*c*), total nitrogen by the Kjeldahl method;[2] organic matter, according to Rather (7); and base exchange capacity using barium chloride with some comparisons of the use of ammonium chloride.

For base exchange determinations 1-gm. or 2-gm. samples were placed in tall 250-ml. pyrex beakers provided with short Pasteur-Chamberlain filter candles and connected to suction flasks. Treatments were repeatedly made with 2 per cent hydrochloric acid, then with neutral half-normal barium chloride. After the samples were washed with warm distilled water until free of chlorides, the barium was displaced with the half-normal neutral ammonium chloride, precipitated and weighed as barium sulfate. As a check, one dozen samples were leached free of chlorides after displacement with the ammonium chloride, and the ammonia was recovered by adding magnesium oxide and distilling at low heat into a saturated solution of boric acid for titration in which brom-phenol blue was used as indicator.

Comparison of methods

A comparison of determinations of base exchange capacity by these two methods is shown in figure 1. Even with careful heating there may be a tendency for nitrogen compounds to decompose during distillation, so that the organic layers of the forest soil profile tend to give slightly higher values with the ammonium method. However, results are in general agreement. The barium chloride treatment is convenient for making a large number of determinations and only a slight tendency toward decomposition was found where successive determinations on the same sample were made.

Physical and microörganic characteristics of forest soil profiles

The forest soil profile samples are briefly described in tables 1 and 2, in which the leading types of vegetation, character of litter, and thickness of layers or

[2] Organic matter determinations were made by Dr. R. E. Stephenson and total nitrogen determinations were made in duplicate by Professor E. F. Torgerson, of these laboratories.

horizons are indicated. These forest soils may be characterized as being generally of a friable, mellow structure, and usually having good drainage. Although the type of vegetation materially affects the soil development, the influence of climate has appeared to have a more profound effect under the conditions studied.

After notes had been taken on visible physical characteristics, the soils were air-dried, ground to pass a screen with 2-mm. perforations, and were then stored in fruit jars.

Absorptiveness for moisture vapor was determined with 1-gm. samples over 3.3 per cent sulfuric acid under standard conditions described by Robinson (8). Absorptiveness usually reaches the maximum in the fermenting layer. A secondary maximum of lesser magnitude occurs in the B horizon or zone of accumulation of the subsoil. Absorptiveness indicates colloidality. Pure inorganic soil colloid is known to absorb approximately 30 per cent moisture;

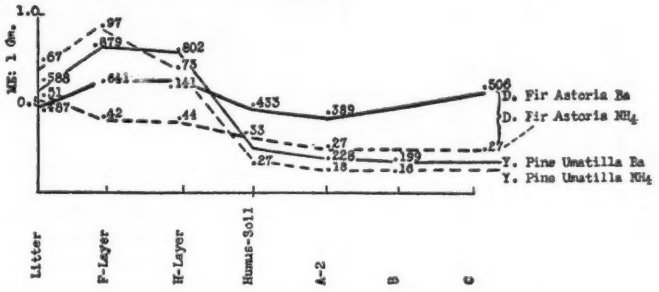

FIG. 1. BASE EXCHANGE CAPACITY OF TWO FOREST SOIL PROFILES
Ba vs. NH_4 method

it has been recently shown (9) that organic soil colloid of low ash content absorbs nearly 45 per cent. The Meyer clay colloid absorbed 27.26 per cent and the peat colloid 40.01 per cent moisture. Perhaps other volatile matter was lost from litter upon drying at 100°C.

Fresh moist samples from profile 3 taken near Astoria were examined and counts made.[3] Results indicate that the maximum number of microörganisms occurs in the fermenting layer. More bacteria and fewer molds were found in the litter.

Chemical characteristics of forest soil profiles

Soil reaction. Colorimetric determinations of reaction value (or pH) of samples from nine forest soil profile layers for which results are given (table 2), show high acidity in the organic layers under conifer forests and conditions of heavy precipitation. The organic acids from conifer litter have caused

[3] By Dr. W. B. Bollen, soil bacteriologist, Oregon Agricultural Experiment Station.

TABLE 1

Absorption of moisture by forest soil profile samples

(Over 3.3 per cent sulfuric acid)

Locality	Smith River, Calif.	Bandon, 4-mile Cr.	Astoria Exp. Sta.	Gresham, 2 mi., S. C.	McKenzie Bridge, 3 mi. E.	Corvallis, 1 mi., S. W.	Sisters, 4 mi. W.	Wanoga Butte	Pioneer Camp, Blue Mts.
Cover	Redwoods	Pine and Fir	Douglas Fir	Douglas Fir	Douglas Fir	Oak	Yellow Pine	Yellow Pine	Pine and Fir
Diameter feet	3–6	1	1–2	1–2½	1–2½	1–2	1–2	1	1–3
Soil Type	Gray-Brown Fine Sandy Loam	Podzolic Very Fine Sandy Loam	Lateritic Silt Loam	Powell Silt Loam	Gravelly Silt Loam	Carlton Silty Clay Loam	Light Medium Silty Loam	Light Medium Loamy Silt	Brown Silty Loam
Absorption—moisture (per cent) in:									
Litter	20.10	19.70	33.31	19.66	25.50	24.20	23.19	34.30	21.09
F-layer	25.88	23.80	13.10	21.11	21.00	31.50	21.45	23.30	24.77
H-layer	23.60	12.80	18.00	12.51	18.50	15.50	10.03	8.70	5.06
H-soil	14.80	7.69	12.00	6.27	11.50	7.70	4.41	4.20	8.29
A_2 horizon	6.70	6.30	8.40	6.12	10.30	7.79	9.01	21.30	10.01
B horizon	4.60	2.50	.....	4.11	10.30	.....	.....	3.63	5.71
C horizon	.....	.....	14.5	.....	.....	.....	.....	.....	.....

TABLE 2

Characteristics of Northwestern forest soil profiles

SOIL PROFILE LAYERS—DEPTH	pH	BASAL EXCHANGE CAPACITY ME: 1 GM.	ORGANIC MATTER* (per cent)	TOTAL† NITROGEN
Cost section				
1. Redwoods—Smith River, California, 1 mile below steel bridge				
Decayed redwood log	5.8	0.530	95.58	0.201
0–2 inches litter, needles, etc.	6.3	0.411	88.96	0.854
2–5 inches F layer	6.7	0.372	58.26	0.623
5–6 inches H layer	6.8	0.156	32.84	0.483
6–12 inches (humus) and soil, fine sandy loam	6.9	0.206	7.76	0.224
12–24 inches gravelly silt loam	6.9	0.094	2.81	0.094
2. Blacklock—Podzolic soil 4 miles South of Bandon, Oregon. Pine and fir				
0–2 inches litter, needles, moss, etc.	6.3	0.334	73.10	1.325
2–2½ inches F layer	6.1	0.336	33.60	0.946
2½–3 inches H layer	6.1	0.271	25.10	0.669
4–12 inches bleached, very fine sandy loam	6.1	0.106	2.74	1.032
16–20 inches iron pan	6.0	0.060	1.09	1.252
20–30 inches raw soil material	6.3	0.034	0.18	0.037
3. Douglas fir—Lateritic soil, Astoria Experiment Station Grove				
0– ¼ inches litter	4.4	0.487	88.10	0.989
¼–1 inches F layer	4.8	0.641	80.50	1.119
1–1½ inches H layer	5.0	0.641	31.80	0.655
1½–2½ inches humus soil, silt loam	5.1	0.433	7.08	0.293
2½–14 inches Soil	5.0	0.389	3.13	0.155
60–66 inches C layer	5.0	0.506	1.30	0.057
Willamette section				
4. Douglas fir—Powell silt loam 2 miles South East of Gresham, Oregon				
0– ¼ inches fir needles, twigs, fern, etc.	5.4	0.175	90.37	1.006
¼–1 inches F layer	6.5	0.166	75.62	1.059
1–2 inches H layer	6.7	0.048	40.86	0.532
2–4 inches humus soil, silt loam	6.8	0.041	14.52	0.136
4–12 inches silt loam	6.9	0.024	2.07	0.099
24–36 inches B layer, silt loam	7.0	.....	0.72	0.038
5. Douglas fir—3 miles above McKenzie Bridge, Oregon. Fir up to 30 inches				
0– ¼ inches litter, needles, twigs, moss	5.4	0.535	67.80	0.894
¼–1½ inches F layer	6.5	0.715	37.20	0.938
1½–3 inches H layer	6.6	0.591	38.20	0.728
3–4 inches humus-soil—gravelly silt loam	6.7	0.101	3.23	0.186
4–18 inches A 2	6.8	0.031	1.53	0.099
30–36 inches B	6.9	0.038	0.81	0.060

TABLE 2—*Concluded*

SOIL PROFILE LAYERS—DEPTH	pH	BASAL EXCHANGE CAPACITY ME:1 GM.	ORGANIC MATTER* per cent	TOTAL† NITROGEN
Willamette section—Concluded				
6. Oak—up to 2 feet, on brownerth near Corvallis, Oregon				
0– ¼ inches litter	6.3	0.300	88.50	1.212
¼– ¾ inches F layer	6.6	0.449	83.85	1.373
¾–1½ inches H layer, mouldy	6.6	0.446	58.88	1.117
2–4 inches humus soil, silty clay loam	6.8	0.254	11.56	0.374
4–12 inches silt loam	6.7	0.144	1.75	0.127
Eastern Oregon				
7. Yellow pine—3 miles west of Sisters, Oregon Yellow pine up to 22 inches				
0– ¼ inches pine needles, chemisa, and grass	4.7	0.380	93.90	0.543
¼–1 inches F layer	5.2	0.447	26.70	0.735
1–1¼ inches H layer	6.3	0.240	24.20	0.428
1¼–4 inches humus soil, medium sandy loam	6.9	0.115	4.37	0.356
12–30 inches B (8 per cent gravel)	6.7	0.062	1.59	0.384
8. Yellow pine—Wanoga Butte, Oregon, 20 miles South West Bend, Oregon				
0– ¼ inches needles, etc.	4.5	0.533	97.00	0.876
¼–3 inches F layer	5.8	0.973	92.78	1.075
3–3½ inches H layer	6.8	0.655	41.40	0.434
4–6 inches soil and humus, medium loamy silt	6.9	0.355	3.88	0.067
6–18 inches pumice soil and roots	6.9	0.248	2.95	0.044
24–36 inches raw pumice	7.0	0.283	1.60	0.030
9. Yellow pine and fir—½ mile South West of Pioneer Camp, Oregon, Trail Hiway, Umatilla County, Trees 1–3 feet in diameter				
0–1 inches needles and twigs, etc.	6.8	0.588	77.20	1.060
1–2 inches F layer	6.8	0.879	69.10	1.177
2–2½ inches H layer	6.8	0.802	43.70	0.861
2½–10 inches humus—soil brown silty loam	6.9	0.313	3.72	0.179
10–24 inches B 1	6.9	0.228	1.37	0.092
24–40 inches B 2, form on rock at 40 inches.	7.0	0.199	0.78	0.059

* Determined by R. E. Stephenson.

† Determined by E. F. Torgerson.

removal of bases from the upper layers of the conifer forests to a greater extent than under deciduous forest cover, which gives less acid litter, having a higher ash content. The more nearly neutral reaction is found in the B layer or zone

of accumulation and the deep subsoil samples are nearly neutral in reaction after the slightly modified soil material of the C horizon is reached.

Organic matter content. The organic matter content of profiles studied decreases with depth and even the litter shows evidence of contamination with inorganic material. Yellow pine needles in the litter of profile 8 show only 3 per cent inorganic matter by the methods used. The Douglas fir litter of profile 5 was nearly one-third inorganic matter. A sample of the deep subsoil or C horizon of profile 3 from near Astoria, taken at a depth of over 5 feet, was below the visible influence of roots and contained 1.3 per cent organic matter, whereas the raw soil material from below the iron pan in the podzol profile, No. 2, contained but 0.18 per cent.

Total nitrogen. Determinations of total nitrogen show the maximum nitrogen content to occur usually in the fermenting layer. It appears that the nitrates formed in the early stages of decomposition are assimilated by and temporarily stored in the bodies of the microörganisms. Thus in this F layer moulds seemed to be more or less prevalent and were especially abundant in the oak soil profile, No. 6. The indicated nitrogen-carbon ratio is rather wide, especially in the case of conifer forest soils. This is related to the more inert nature of the conifer litter which is unfavorably low in nitrogen and earthy bases for prompt decomposition.

Base exchange capacity. Base exchange capacity is reported in millequivalents per gram sample. It is interesting to note that the nearly fresh litter of various forest types manifests base exchange capacity. The maximum base capacity is usually found in the fermenting layer or where there is a mixture of about three-fourths humified organic matter with one-fourth inorganic material. The value is only slightly lower in the humified layer as a rule. Forest profile samples from the semi-arid sections of eastern Oregon have been less leached and show higher base capacities in their surface layers than do those from the humid coastal region where leaching and some degeneration have occurred.

Base exchange capacity of artificial mixtures

To study further the effects of mixtures on base exchange capacity, organic soil colloids isolated from neutral peat soil of low ash content were mixed with colloids derived from Aiken silty clay loam. These materials were mixed in proportions varying by 20 per cent intervals, with resulting base capacities as presented in figure 2. With this mixture the maximum base exchange capacity was realized with 80 per cent peat and 20 per cent clay.

In a second experiment, using the same peat colloid in mixtures with colloid from Meyer clay adobe from near Medford, a maximum base capacity was obtained with 60 per cent peat and 40 per cent clay colloid. The Meyer clay colloid had a reaction value of pH 8, or was slightly alkaline, and had a much higher base exchange capacity when used alone than did the acid Aiken colloid (pH 6.2). There is a striking similarity between the base exchange capacity

of natural mixtures such as occur in sedimentary peat layers or in the humified layers of forest soil profiles and that of these artificial mixtures, as will be noted by comparing figures 1 and 2.

The author recently studied the effect of mixing peat colloid with clean quartz sand, using 0.1 per cent, 0.5 per cent, and 1 per cent proportions of peat colloid. The base exchange capacity per gram of colloid increased where it was more thinly spread over the sand grains. The experiment was repeated using lignin isolated from fiber flax with similar results. These experiments (9) indicate that the base exchange activity may increase in the case of lignin down to one oriented molecular layer. Great base exchange capacity has also been found in connection with these studies of organic colloid derived from decomposing sweet clover straw in the presence of lime and mixed with inorganic soil colloid.

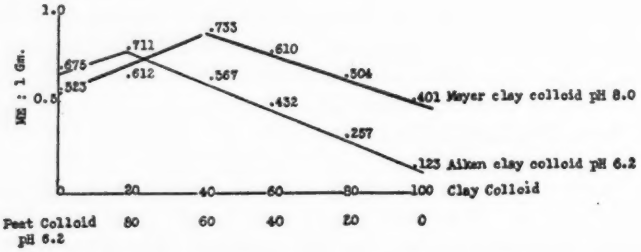

FIG. 2. BASE EXCHANGE CAPACITY OF PEAT AND CLAY COLLOID MIXTURES

DISCUSSION

The evidence herein presented is to the effect that a mixture of organic soil colloid and clay colloid has a very high base adsorbing capacity. This correlates with the high base exchange capacity of sedimentary peat layers and humifying layers of forest litter and brings out the fundamental rôle of organic soil colloids in soil conservation. The activity of earthworms in neutral brownerths or in humified layers of deciduous forest soils aids in the formation of an intricate mixture of organic and inorganic colloidal matter. The nitrogen is concentrated in the organic colloid fraction. Lunt (4) has recently reported finding that approximately 40 to 45 per cent of the exchange calcium in the whole forest soil profile was in the humic portion and that as much as 61 per cent of the total calcium in the humified layer was in exchange form. In other words, as the lignin concentration increases, the exchange portion of the calcium increases.

It appears to be easier to build up and keep up base exchange capacity of a forest soil that is well supplied with calcium, contains some deciduous growths (11), and is reasonably free from leaching. This helps explain the occurrence of black earth in northern semi-arid continental prairies. The presence of

lime facilitates building up of organic matter in arid soils when brought under irrigation and rotated with legumes, as recently noted (10). Decaying organic matter seems to increase nutrient supplying power of soil as to phosphate (6), iron (2), and potassium (3), as well as other bases and also nitrate.

The forest litter helps maintain tilth and conserve moisture with no cultivation. Acid conifer litter is resinous, low in nitrogen, and also slow to decay. It yields an acid extract which leaches out the bases, giving rise to the bleached gray, podzolic layer. These bases are removed partly in colloidal solution and carried down to a zone of more neutral reaction where precipitation occurs. The humus is believed to form a protecting cover to colloidal iron and hold it in colloidal solution during removal to the lower layer where it precipitates and helps form the hardpan. The analyses show some accumulation of organic matter in the coffee-brown pan formation of the Blacklock soil.

Logged off land in the humid Northwest is often acid and in need of lime, which will precipitate or render toxic substances unharmful. Decaying manure reinforced with superphosphate may also be expected to favor a reasonable rate of decomposition and increased productiveness.

Results obtained should be of value in economy of soil organic matter and conservation of base exchange capacity or protection against soil degeneration. The high nutrient supplying power of the humified layers of forest soils is further supported by the mass of feeding roots observed therein. The character and amount of forest litter and the trend of humification of this material under the climatic conditions obtaining have a profound effect upon soil development and continued soil productiveness.

SUMMARY

The great forest soils of the Pacific Northwest have been little studied. Their organic layers yield materials at different stages of decomposition and with different proportions of mineral earth intermixed. Studies made with nine forest soil profile layers usually show maximum absorptiveness for moisture vapor or colloidality in the fermenting layers. Here the maximum numbers of microörganisms were also found. The maximum base exchange capacity was generally found in nearly neutral fermenting or largely humified organic layers containing approximately 75 per cent organic matter. A similar type of adsorption curve was obtained whether barium or ammonium ion was the saturating agent. Artificial mixtures of organic and inorganic soil colloids varied by 20 per cent interval, show a corresponding base adsorption curve. That surface concentration may help account for this was recently suggested in connection with similar results obtained with natural colloids separated from sedimentary peat layers (9). Marked activity of earthworms in neutral or calcareous soil abundantly supplied with decaying ligneous organic matter favors formation of this desirable intimate mixture of organic and inorganic soil colloids. Feeding roots are massed in the forest soil just below this organic

layer. These results should be of value in composting, in the use of litter or green manure, as well as in conservation of base exchange capacity of soil against degeneration.

REFERENCES

(1) ALBEN, A. O. 1930 Reactions of electrodialized humus and bentonites and application of the method. *Jour. Amer. Soc. Agron.* 22: 703.

(2) BURK, D., ET AL. 1931 Relation between iron, humic acid, and organic matter. *Science* 74: 522.

(3) HEDDEN, W. P. 1931 Effects of clover and alfalfa in rotation: II–IV. Colo. Agr. Exp. Sta. Bul. 263, 264, and 265.

(4) LUNT, H. A. 1931 Discussion—*Proc. Amer. Soil Survey Assoc.*, 13: 24.

(5) MCGEORGE, W. T. 1930 The base exchange properties of organic matter in soils. *Ariz. Agr. Exp. Sta. Tech. Bul.* 30: 182.

(6) MCGEORGE, W. T., AND BREAZEALE, J. F. 1931 Relation of phosphorus availability, soil permeability and carbon dioxide to fertility of calcareous soils. Ariz. Agr. Exp. Sta. Tech. Bul. 36.

(7) RATHER, J. B. 1917 An accurate loss-on-ignition method for the determination of organic matter in soils. Ark. Agr. Exp. Sta. Bul. 140.

(8) ROBINSON, W. O. 1922 The absorption of water by soil colloids. *Jour. Phys. Chem.* 20: 647.

(9) POWERS, W. L. 1932 Characteristics of organic colloids in peaty soils. *Jour. Agr. Res.* 44: 97.

(10) POWERS, W. L., AND LEWIS, R. D. 1930 Nitrogen and organic matter as related to soil productivity. *Jour. Amer. Soc. Agron.* 22: 825.

(11) TOUMEY, J. W. 1928 Foundations of Silviculture upon an Ecological Basis, p. 100–129. J. Wiley & Sons, New York.

THE DISTRIBUTION OF POTASSIUM IN DECIDUOUS ORCHARD SOILS IN CALIFORNIA

OMUND LILLELAND[1]

University of California

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The scorching of foliage of prune (*Prunus domestica* var. Agen) trees in parts of the Sacramento Valley seems to be associated to some extent with the potassium content of the soil. It seemed desirable to study the potassium relationships as they exist in various depths in areas where scorching does not appear and to compare these with soils in which prune trees show this trouble.

Analyses of soils in 21 prune orchards[2] in various sections of the state have, therefore, been made in the pomology laboratory at the University Farm, Davis. The approximate locations of these orchards and the representative soil types are listed in table 1. The soils are all of alluvial origin and exhibit no marked changes in texture in the top 4 feet.

It soon became evident from the analyses of these soils that the water-soluble and replaceable potassium varied with the depth of sampling. A definite reduction in these two potassium fractions with increased depth of sampling has been found in practically all the soils studied to date. The data listed in table 2 are typical of the changes in the water-soluble potassium. The reduction is most marked in the soils having the higher amounts. Although a great variation exists in the water-soluble potassium in the surface foot of the various orchards, the range of concentrations is narrowed with increased depth.

A study of table 3 reveals that the reduction in replaceable potassium in the deeper soil is marked, but not quite so rapid as that reported for the water-soluble form. Soils from the Los Molinos 3 and 4 and Vina 1 orchards seem to maintain their high concentrations of replaceable potassium in the third and fourth feet to a greater degree than one would be led to expect from the water-soluble fraction of these soils. The relationship between water-soluble and replaceable potassium does not seem to be one of simple hydrolysis, as suggested by Magistad (4). Samples identical in their replaceable potassium content but taken at varying depths exhibit varying contents of water-soluble potassium. Generally the surface samples show proportionally a greater water-soluble fraction than do the deeper samples.

[1] I am indebted to Mr. C. J. Hansen and Mr. E. A. Wilkins for their assistance with the chemical analyses.

[2] Subsequent data have been obtained from 10 additional orchards and are in accord with the results presented here.

TABLE 1

The prune orchards in which soil samples were studied

ORCHARD	COUNTY	OWNER	SOIL TYPE
1. Los Molinos 1	Tehama	Lindauer	Sacramento fine sandy loam
2. Los Molinos 2	Tehama	Mundorff	Sacramento silty clay loam
3. Los Molinos 3	Tehama	Smith	Sacramento silt loam
4. Los Molinos 4	Tehama	Barton	Sacramento silty clay loam
5. Vina 1	Tehama	Woolsey	Vina fine sandy loam
6. Richfield 1	Tehama	Aasen	Elder silt loam
7. Chico 1	Butte	Canfield	Farwell fine sandy loam
8. Chico 2	Butte	McVay	Farwell loam
9. Chico 3	Butte	Peters	Farwell loam
10. Chico 4	Butte	Sypher	Vina fine sandy loam
11. Colusa 1	Colusa	Wolflin	Sacramento silty clay loam
12. Colusa 2	Colusa	Taft	Sacramento silty clay loam
13. Grimes 1	Colusa	Faxon	Sacramento fine sandy loam
14. Davis	Yolo	Univ. Farm	Yolo loam
15. Gilroy 1	Santa Clara	Barbari	Yolo silt loam
16. Paradise Valley 1	Santa Clara	Ward	Vina gravelly loam
17. Morgan Hill 1	Santa Clara	Hansen	Yolo silt loam
18. Saratoga 1	Santa Clara	Anderson	Yolo gravelly loam
19. Cupertino 1	Santa Clara	Wiesendanger	Yolo clay loam
20. Berryessa 1	Santa Clara	Moody	Yolo gravelly clay loam
21. Saratoga 2	Santa Clara	Payne	Yolo loam

TABLE 2

Water-soluble (1:1) potassium content of dry soil in relation to the depth of sampling

ORCHARD	WATER-SOLUBLE POTASSIUM			
	First foot	Second foot	Third foot	Fourth foot
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1. Los Molinos 1	2.6	1.6	1.4	1.4
2. Los Molinos 2	5.6	2.8	2.2	....
3. Los Molinos 3	12.0	6.3	4.6	3.7
4. Los Molinos 4	10.4	8.8	8.3	9.0
5. Vina 1	8.0	10.4	8.2	....
6. Richfield 1	10.0	6.2	3.5	1.5
7. Chico 1	1.2	0.4	0.4	0.2
8. Chico 2	2.1	0.7	0.9	0.8
9. Chico 3	6.7	2.1	1.2	0.8
10. Chico 4	16.3	10.8	5.8	1.8
11. Colusa 1	4.3	2.3	2.3	1.5
12. Colusa 2	1.9	0.9	1.7	1.0
13. Grimes 1	49.5	49.8	40.6	14.3
14. Davis	5.3	2.2	1.2	1.2
15. Gilroy 1	13.7	5.2	1.7	1.2
16. Paradise Valley 1	5.3	3.0	2.3	1.9
17. Morgan Hill 1	7.4	1.6	0.9	0.8
18. Saratoga 1	9.7	5.7	2.7	2.0
19. Cupertino 1	14.2	9.0	2.8	1.1
20. Berryessa 1	7.2	3.3	1.5	1.7
21. Saratoga 2	6.6	2.0	1.0	1.2

Determinations of "active" potassium in these soils using 0.2 *N* nitric acid as described by Fraps (1) give results not far different from those obtained by replacement with the ammonium ion. A comparison of a few values in table 4 led to the conclusion that the replaceable and active fractions differed very slightly and that a continued study by this method would only be a duplication of the ammonium acetate extraction.

It was thought that such concentration gradients as were found in the water-soluble and replaceable potassium contents might be proportionally reflected in the analyses for total potassium. Fusion analyses of representative soils

TABLE 3

Replaceable potassium content of dry soil in relation to depth of sampling

ORCHARD	REPLACEABLE POTASSIUM			
	First foot	Second foot	Third foot	Fourth foot
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1. Los Molinos 1	170	140	140	130
2. Los Molinos 2	135	88	51	...
3. Los Molinos 3	650	405	342	350
4. Los Molinos 4	661	574	491	426
5. Vina 1	437	356	349	334
6. Richfield 1	173	120	105	90
7. Chico 1	110	86	84	52
8. Chico 2	134	84	74	49
9. Chico 3	434	244	128	97
10. Chico 4	985	950	497	183
11. Colusa 1	293	138	118	100
12. Colusa 2	141	102	103	116
13. Grimes 1	1,240	1,180	1,027	489
14. Davis	325	180	153	124
15. Gilroy 1	461	270	143	119
16. Paradise Valley 1	341	174	175	143
17. Morgan Hill 1	383	221	168	142
18. Saratoga 1	355	171	150	140
19. Cupertino 1	625	466	259	140
20. Berryessa 1	268	135	116	126
21. Saratoga 2	284	144	108	92

show no such reduction in the total potassium content of the soil with increase in depth of sampling. Only a few determinations were made, but these results, listed in table 5, indicate that this fraction cannot account for the decreases observed with the water-soluble and replaceable potassium.

It appears as if these soils, though markedly different in their water-soluble and replaceable potassium content at the surface, might all be found to be very similar at some depth beyond 4 feet. A few samples have been taken to a depth of 8 feet and suggest that such a minimum concentration is attained and maintained in the deeper samples (table 6).

Although sodium and potassium are closely allied chemically it has been generally established that potassium is more readily retained by the soil. Only seven of the soils have been analyzed for sodium and the analyses show no evidence of an accumulation of this particular cation at the surface. The exchangeable sodium either remains fairly constant or increases in the deeper samples. Prune trees growing in Colusa 2 orchard, which is particularly high

TABLE 4

Replaceable and active potash content of dry soil in relation to the depth of sampling

ORCHARD	DEPTH	POTASSIUM CONTENT		
		Replaceable	Active	Difference
	feet	*p.p.m.*	*p.p.m.*	*p.p.m.*
Chico 1	1	191	159	−32
	2	89	96	+7
	3	81	74	−7
	4	85	65	−20
Chico 1	1	434	468	+34
	2	240	264	+24
	3	180	190	+10
	4	154	152	−2
Grimes 1	1	1,242	1,455	+213
	2	1,185	1,400	+215
	3	1,027	1,133	+106
	4	489	564	+75
Gilroy 1	1	461	500	+39
	2	270	304	+34
	3	143	160	+17
	4	119	....	....
Morgan Hill	1	383	448	+65
	2	221	250	+29
	3	168	197	+29
	4	142	.....	.....
Paradise Valley 1	1	341	302	−39
	2	174	192	+18
	3	175	167	−8
	4	143	151	+8

in sodium, are showing some distress. Alfalfa and grain crops adjacent to this orchard, however, do well.

Analyses were also made on the water-soluble calcium and magnesium content of soil in relation to depth of sampling and the data concerning these two constituents show no definite trend of change in the concentration of either of these two ions with increase in depth.

TABLE 5

Total potassium content in relation to depth of sampling

ORCHARD	SAMPLE NUMBER	DEPTH	K
		feet	*per cent*
Grimes 1 (Faxon)	390	1	1.47
	391	2	1.36
	392	3	1.35
	393	4	1.23
Chico 2 (Canfield)	436	1	0.92
	437	2	0.82
	438	3	0.72
	439	4	0.79
Chico 2 (Canfield)	472	1	1.02
	473	2	0.93
	474	3	0.96
	475	4	0.95
Gilroy 1 (Barbari)	197	1	1.92
	198	2	2.02
	199	3	1.83
	200	4	1.79
Morgan Hill 1 (Hansen)	201	1	2.10
	202	2	2.16
	203	3	1.96
	204	4	1.82
Paradise Valley 1 (Ward)	205	1	0.89
	206	2	0.78
	207	3	0.89
	208	4	1.04

TABLE 6

Replaceable potassium content of dry soil

ORCHARD	DEPTH OF SAMPLING							
	First foot	Second foot	Third foot	Fourth foot	Fifth foot	Sixth foot	Seventh foot	Eighth foot
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Chico 3	430	240	130	100	90	85	85	80
Chico 4	350	130	85	80	70	75	75	70
Chico 4	400	155	80	80	85	70	75	60
Chico 4	505	330	235	170	140	115	85	80

Similarly the analyses on the replaceable calcium and magnesium content of soil in relation to depth of sampling[3] show that the replaceable calcium in

[3] No corrections have been made for Ca and Mg that may be dissolved by the ammonium acetate solution.

most of these soils, except those from the Berryessa and Richfield orchards, remains relatively constant with a tendency to be slightly lower in the deeper samples. Replaceable magnesium in these orchard soils, like calcium, shows no distinct trend to increase or decrease. The deeper samples as a rule show a slightly higher magnesium content.

DISCUSSION

The distinct reduction in the potassium content of these orchard soils in the deeper samples is so general and consistent as to cause one to speculate on the cause of this concentration gradient. Any solution offered to account for the higher potassium content of the surface soils must also take into consideration its chemically allied cation, sodium, whose concentration either remains fairly constant or tends to increase in the lower depths. It must also be in accord with the data obtained concerning the divalent cations calcium and magnesium whose concentrations in the various depths studied remain relatively uniform. Undoubtedly many factors are involved and further analyses of these soils would be helpful in formulating a theory, but such are not within the scope of the present investigation.

A partial explanation that does not seem to be contradictory to the present data is the influence of vegetation on the chemical composition of the surface soil. Plant residues contain relatively large amounts of potassium. The computed average composition of 24 varieties of grasses as originally determined by Wolff (6) indicates the predominance of potash. Potassium may be absorbed by plants from the deeper soil layers. Upon the death and decay of these plants, the potassium present in their tissues augments the replaceable potassium in the surface soil.

Average composition of 24 varieties of grasses (hay and straw)

	Per cent dry weight
K_2O	1.49
Na_2O	0.20
CaO	0.90
MgO	0.29

It seems probable that under conditions of limited rainfall such as are experienced in the prune growing sections of California, a potassium gradient could have been established and maintained by native vegetation.

Analyses of soils from dry yards adjacent to some of these orchards seem to confirm this hypothesis. These dry yards are areas in which the fruit is spread on wooden trays to dry and which are generally allowed to grow to native grasses and weeds or often are planted to cereal hays which are cut just prior to the fruit harvest. Growers assured me that no tree crops had ever been planted in these sampled areas nor had any manure ever been applied. Yet these soils show potassium gradients similar to those found in the orchards. The data on these dry yard areas are presented in table 7.

There may be several factors operating to preclude the establishment of like concentration gradients of the other cations. Potassium is a specially favored element in that it is most readily fixed in the soil and may replace calcium and other cations. The ease with which it enters into the replaceable complex may account in some degree for the absence of marked changes in concentration of these other cations with depth of sampling.

The solubility of calcium and magnesium carbonates and other salts containing these cations may be great enough to obscure any gradient in concentration produced by plant residues. Kelly and Arany (2) report the existence of relatively large amounts of Ca and Mg as compounds other than replaceable and carbonate forms soluble in ammonium chloride solution.

TABLE 7

Potassium content of dry soil from dry yard areas adjacent to prune orchards

DRY YARD	WATER-SOLUBLE K (1-1)				REPLACEABLE K			
	First foot	Second foot	Third foot	Fourth foot	First foot	Second foot	Third foot	Fourth foot
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Saratoga 2 (Payne)	12.8	4.6	1.9	2.1	458	261	131	143
Chico 5 (Stiles)	10.5	3.1	2.5	1.8	572	320	274	157

SIGNIFICANCE IN SOIL SAMPLING

Recent studies indicate that the fraction of the soil potassium that is replaced by the ammonium ion is available to plants (5, 7). In most soils this fraction is a measure of the soil productivity as regards potassium. Professor Hoagland of the plant nutrition division has, however, called my attention to a soil which is low in replaceable potassium and yet is able to maintain an adequate supply of potassium from other types of minerals present in this particular soil. Although the decreases in available potash content of soils with increase in depth may not be of importance to annual crops, they may have some significance in the study of the nutritional requirements of deciduous fruit trees. Certain experimental field studies in which potash was distributed deep in the soil (3) in comparison with potash applied on the surface indicate that greater response is obtained with the deeper distribution. With differences existing between the surface foot and those deeper down and with our present lack of knowledge as to the relative importance of the various depths of soil as a source of inorganic nutrients for deciduous fruit trees, it would seem desirable to examine orchard soils more critically than a composite or surface sample will permit.

REFERENCES

(1) FRAPS, G. S. 1912 The active potash of the soil and its relation to pot experiments. Tex. Agr. Exp. Sta. Bul. 145.

(2) KELLY, W. P., AND ARANY, ALEX 1928 The chemical effect of gypsum, sulfur, iron sulfate, and alum on alkali soil. *Hilgardia* 3: 393–420.

(3) McKINNON, L. R., AND LILLELAND, O. 1931 A method of application designed to insure proper distribution of fertilizers in field trials with fruit trees. *Soil Sci.* 31: 407–410.

(4) MAGISTAD, O. C. 1928 The hydrolysis of sodium and potassium zeolites with particular reference to potassium in the soil solution. Ariz. Agr. Exp. Sta. Tech. Bul. 22.

(5) MARTIN, J. C. 1929 The effect of crop growth on the replaceable bases in some California soils. *Soil Sci.* 17: 123–136.

(6) THORPE, T. E. 1921 Dictionary of Applied Chemistry, vol. 1, p. 307. Longmans, New York.

(7) WHEETING, L. C. 1930 A study of methods for determining available potassium in soils. *Soil Sci.* 29: 1–22.

DETERMINATION OF THE RATE OF DECOMPOSITION OF ORGANIC MATTER UNDER FIELD CONDITIONS[1]

M. B. STURGIS

Louisiana State University

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In studying the effects of the decomposition of sugar cane trash on the total and available nitrogen of the soil,[2] it was found that as the carbohydrate fraction of the organic material added disappeared the available nitrogen in the soil tended to increase. Practically all of the soil organic matter is found in the lignin-humus, protein, and carbohydrate fractions, and the total soil organic matter can be estimated from the total organic carbon content (6). This condition makes possible the calculation of the carbohydrate fraction by difference. Since the greater part of sugar cane trash is composed of carbohydrates, it was desirable to develop a more direct method of measuring the rate of the decomposition of the carbohydrate fraction of the trash in the soil.

EXPERIMENTAL METHODS

A series of closely controlled 1/2,000-acre field plats on Sharkey silty clay loam were treated with sugar cane trash and ammonium sulfate and kept bare of crops. There were four plats under each treatment and the treatments were as follows:

1. Check, no treatment
2. 12 tons cane trash and 80 pounds nitrogen per acre
3. 12 tons cane trash and 40 pounds nitrogen per acre
4. 12 tons cane trash per acre

The first application of trash and nitrogen was made in March, 1930. The second application was made on October 27, 1930, and the data given here are from the second application. The trash was chopped and then the trash and ammonium sulfate were applied to the soil and mixed with it to a depth of 5 inches.

Samples were taken from the first 6 inches. The soil at the first sampling after being treated with trash had a volume weight of 1.21.

Total nitrogen was determined by the Gunning-Hibbard method.

[1] Published with the approval of the director of the Louisiana Agricultural Experiment Station.

[2] STURGIS, M. B. The effect of additions of nitrogen on the decomposition of sugar cane trash under field conditions. Unpublished data Louisiana Agricultural Experiment Station, 1931.

Organic carbon was determined by the dry combustion method.

Ether extract was determined by extracting the dry material in a Soxhlet for 8 hours. Fifty-gram samples of the soil material were used.

Alcohol extract was determined on the material after the ether extraction by treating it in a Soxhlet for 8 hours with 95 per cent alcohol. The alcohol extract was dried and weighed as in an ether extraction.

Lignin and lignin carbon were determined on the material after the ether and alcohol extractions by a combination of the methods of Dore (2) and Schwalbe (4). A sample weight that would give a residue of not more than 0.10 gm. of carbon was taken. Three samples were run. The nitrogen content of the lignin fraction was run and calculated to protein, the ash content was determined, and the lignin was calculated free of protein and ash. In the determination of the carbon content of the lignin fraction two samples were run. The carbon content of one and the nitrogen content of the other were found. Then the carbon content of the lignin was calculated free of the protein carbon.

Reducing sugars were determined on the carbohydrate material put in solution and hydrolyzed in the treatment for the isolation of the lignin fraction. Reducing sugars were also run on the alcoholic extract of fresh trash. All reducing sugars were determined as anhydrous dextrose. By a comparison of the results of the proximate method of analysis used in this work with the results of the more exact methods of Brown and Blouin (1), it will be noted that most of the error in the proximate method falls in the carbohydrate fraction. This is due not so much as might be suspected to the fact that the different sugars have different reducing values and that the non-reducing sugars are not determined, for dextrose makes up more than 60 per cent of the sugars, with xylose, arabinose, levulose, and galactose being represented in correspondingly small amounts as the only other sugars occurring in appreciable quantities. The greatest error introduced into the determination of all the carbohydrates by hydrolysis to reducing sugars is due to either incomplete hydrolysis or decomposition of hydrolyzed products or both. Strong acid and high temperatures will cause decomposition and humification of the hydrolyzed material (5), which will be reflected in low yields of reducing sugars and too high results for lignin or lignin-humus.

The reducing sugars from the hydrolysis of the carbohydrates in trash were determined on the filtrate from the lignin residue. But it was found that for soil material a separate sample had to be taken. The following method gave somewhat low but consistent results:

The soil material remaining after the ether and alcohol extractions was dried and a 20-gm. sample taken. The sample was placed in a 500-cc. glass stoppered Erlenmeyer flask and moistened with 5 cc. of 18 per cent hydrochloric acid. Then 25 cc. of 72 per cent sulfuric acid was pipetted into the flask, care being taken to wash all the soil material to the bottom; the glass stopper was lubricated with the sulfuric acid, placed in the flask, and weighted down against the pressure. The pressure of HCl will be from 20 to 30 mm. above atmospheric pressure. The flask was rotated to mix completely the acids and sample and quickly placed in a water bath at 20°C. After 24 hours, the contents of the flask were diluted with 300 cc. of water and boiled under a reflux condenser for 1 hour. The solution was filtered through a Gooch

crucible and the lignin-humus residue washed free of acids. The iron, aluminum, and manganese were precipitated from the filtrate with sodium carbonate and sodium hydroxide. Two precipitations were necessary. Sodium carbonate was added to the solution until precipitation began, then a 10 per cent solution of sodium hydroxide was added to bring the solution up to pH 6.0. The mixture was made up to 400 cc. and filtered through a Büchner funnel provided with a filter paper and asbestos mat. Sodium hydroxide solution was then added to the filtrate until the mixture was very slightly alkaline, pH 9.0. The volume of the alkaline solution used in the second precipitation was noted and added to the original volume of 400 cc. in the calculation. A second filtration was made using a new filter paper and mat. Reducing sugars were then determined as anhydrous dextrose on the clear, slightly alkaline filtrate by the method of Lane and Eynon (3) for the determination of reducing sugars by means of Fehling's solution with methylene blue as an internal indicator. Very low concentrations required that the method be modified for the use of 2 cc. of Fehling's solution and that a table of factors and dextrose values be made for the conditions of the determination.

The soil used in this experiment did not contain sufficient higher oxides of manganese to liberate chlorine from the hydrochloric acid, but the hydrolysis of carbohydrates in soils that contain higher oxides which will liberate chlorine from hydrochloric acid can be better accomplished by the use of sulfuric acid only.

A test of the method on 1-gm. samples of material which contained 99.3 per cent cellulose showed that it was possible to recover 97.8 per cent of the cellulose as dextrose. There was a residue of 2.4 per cent of the sample of which a part was degraded and a part not hydrolyzed. In studying the hydrolysis of the same material in the presence of soil, it was possible to recover only 91.1 per cent of the cellulose as dextrose.

The percentage of the added carbohydrates that had decomposed was calculated from the difference between the reducing sugars present in the soil at a given time and the sum of the reducing sugars in the soil at the application of the trash and the expected increase from the addition of the trash, the expected increase from the trash being considered as 100 per cent.

RESULTS

The results given in tables 1 and 3 following the various treatments are the averages from samples taken from the four plats under each treatment.

Data in table 3 indicate that there was a very appreciable disappearance of the carbohydrate fraction as reducing sugars within the first month following

TABLE 1

Organic matter in the soil before application of the cane trash and nitrogen

Results are on the basis of dry soil

DATE	TREATMENT	TOTAL N	ORGANIC C	N:C	LIGNIN-HUMUS C	REDUCING SUGARS
		per cent	*per cent*		*per cent*	*per cent*
October 27, 1930	1	0.1053	1.074	1:10.2	0.493	0.308
	2	0.1118	1.165	1:10.4	0.535	0.352
	3	0.1110	1.167	1:10.5	0.538	0.353
	4	0.1093	1.139	1:10.4	0.524	0.341

TABLE 2

*Composition of the cane trash applied on October 27**

Moisture is on the basis of moist field weight. Other determinations are on the dry basis.

	PER CENT
Moisture	70.55
Ash	9.92
Total N	0.82
Total C	41.95
Ether extract	2.57
Alcohol extract (less reducing sugars in alcohol extract)	5.10
Lignin (62.8 per cent C)	13.23
Total reducing sugars (as anhydrous glucose)	63.45

* Had it been possible to sample the soil immediately after applying the trash the percentages of nitrogen, carbon, and reducing sugars in the soil would have increased 0.0035, 0.180, and 0.272 respectively.

TABLE 3

Organic matter in the soil after the application of the cane trash and nitrogen

Results are on the basis of dry soil

DATE	TREATMENT	TOTAL N	ORGANIC C	N:C	ETHER AND ALCOHOL EXTRACT	LIGNIN HUMUS C	REDUCING SUGARS	ADDED CARBOHYDRATES DECOMPOSED
		per cent	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
November 25, 1930	1	0.1054	1.062	1:10.1	0.037	0.488	0.307	.....
	2	0.1200	1.384	1:11.5	0.050	0.613	0.513	40.7
	3	0.1157	1.339	1:11.6	0.056	0.626	0.526	36.4
	4	0.1114	1.302	1:11.7	0.054	0.612	0.512	37.1
January 22, 1931	1	0.1060	1.058	1:10.0	0.037	0.483	0.306	.....
	2	0.1188	1.311	1:11.0	0.050	0.575	0.465	57.7
	3	0.1162	1.304	1:11.2	0.052	0.597	0.491	49.2
	4	0.1126	1.306	1:11.6	0.046	0.612	0.508	38.6
March 11, 1931	1	0.1060	1.063	1:10.0	0.042	0.490	0.278	.....
	2	0.1155	1.229	1:10.6	0.052	0.547	0.401	81.2
	3	0.1157	1.271	1:11.0	0.046	0.574	0.413	77.9
	4	0.1142	1.294	1:11.3	0.051	0.597	0.453	58.8
June 12, 1931	1	0.1040	1.044	1:10.0	0.041	0.464	0.275	.....
	2	0.1173	1.254	1:10.7	0.046	0.579	0.371	92.3
	3	0.1163	1.233	1:10.6	0.050	0.581	0.388	87.1
	4	0.1140	1.226	1:10.8	0.041	0.572	0.402	77.6
August 1, 1931	1	0.1049	1.048	1:10.0	0.039	0.460	0.271	.....
	2	0.1159	1.180	1:10.2	0.050	0.501	0.324	109.6
	3	0.1129	1.164	1:10.3	0.048	0.521	0.324	110.7
	4	0.1119	1.153	1:10.3	0.044	0.520	0.338	101.1

the application of the trash and ammonium sulfate. The results from the first sampling are not entirely consistent and the discrepancies can be partly explained by the fact that it is very difficult to get a truly representative sample before the trash is largely decomposed. Later results show that the carbohydrate fraction decomposes much more readily than the lignin-humus and protein fractions and that the addition of nitrogen with trash increases the rate of the decomposition of the carbohydrates. A slightly greater amount of carbohydrate than was added through the trash disappeared from the plats that received trash only in the course of the experiment. This extra decomposition of the carbohydrates already in the soil before the application of the trash is also indicated from the fact that the nitrogen-carbon ratio for treatment 4 was lower at the end of the experiment than at the time the trash was applied (table 1). Decomposition of the carbohydrates and the narrowing of the carbon-nitrogen ratio are in good agreement with each other and show that all the trash applied had decomposed to a greater degree or to a more narrow carbon-nitrogen ratio than existed in the soil when the trash was applied.

The lignin-humus fraction of the trash decomposed slowly but it did not accumulate as did the protein fraction.

The protein or nitrogen fraction was the most resistant part of the soil organic matter and because of the synthetic processes of the microörganisms involved in the decomposition of a material which contained a small amount of nitrogen and a great amount of carbohydrates, the nitrogen fraction accumulated in the soil.

CONCLUSIONS

Since the various fractions of organic materials decompose at different rates in the soil, it is important to be able to determine by as direct method as possible changes in the amounts of the major fractions of the soil organic matter.

A method for the determination of the carbohydrates in the soil as reducing sugars has been developed and found to give good results in measuring the rate of the decomposition of the carbohydrate fraction of sugar cane trash in the soil.

REFERENCES

(1) Brown, C. A., Jr., and Blouin, R. E. 1907 The chemistry of the sugar cane and its products in Louisiana. La. Agr. Exp. Sta. Bul. 91.

(2) Dore, W. H. 1920 The proximate analysis of hardwoods. Studies on *Quercus agrifolia*. *Jour. Indus. and Engin. Chem.* 12: 984–987.

(3) Lane, J. H., and Eynon, L. 1923 Determination of reducing sugars by means of Fehling's solution with methylene blue as an internal indicator. *Jour. Soc. Chem. Indus. Trans.* 42: 32T–37T.

(4) Schwalbe, H. 1925 Eine neue Method zur Bestimmung des Lignins. *Papier Fabrik.* 23: 174–177.

(5) Sherrard, E. C., and Harris, E. E. 1932 Factors influencing properties of isolated wood lignin. *Jour. Indus. and Engin. Chem.* 24: 103–106.

(6) Waksman, S. A., and Stevens, K. R. 1930 A critical study of the methods for determining the nature and abundance of organic matter. *Soil Sci.* 30: 97–116.

THE SOIL AS A HABITAT FOR GROWTH OF GREEN ALGAE

C. E. SKINNER

University of Minnesota[1]

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It has been known for a long time that the soil harbors blue-green and green algae. Until recently, however, it has been assumed that the algae were chance invaders, and that they multiplied only under conditions of extreme moisture and only on the surface. The early work of Esmark (4), Peterson (9), and Robbins (10) demonstrated a large number of species in soil. Later work by Bristol Roach (1, 3) and by Moore and associates (7, 8) in normal soil and in soil dried for a half century also showed that algae were found in deep as well as surface soil in large numbers. Bristol Roach found in many soils more than 100,000 cells per gram (2, 3).

The fact that algae are found in soil deep enough to prevent any possible nutrition by photosynthesis should not cause surprise, inasmuch as it has been known that many species of green algae are able to grow saprophytically in complete darkness for years without a diminution of their activities, and with the production of abundant chlorophyll, provided sufficient energy in the form of available organic nutrients is present.

That algae actually exist in the soil in an active non-resting state was first demonstrated by Bristol Roach (2, 3) using a modification of McLennan's (6) ingenious technique for "counting" active fungi in the soil. Bristol Roach found that rapid drying of the soil destroyed the vegetative cells of certain species of green algae without destroying the resting cells. Her method (3)

"consists in counting the total number of individuals in one part of a well-mixed freshly gathered soil sample by means of a cultural dilution method, and in counting the number of resting or resistant forms in a second part of the same sample after it has been subjected to rapid desiccation by means of a stream of dried sterile air at laboratory temperature, the vessel containing the soil being immersed in a water bath at 35°C. so that the conditions should approximate those of a dry wind on a hot day. A comparison of the two counts gives some idea of the proportion of vegetative cells to resting cells. It has been found that the different species do not all react in the same manner to this treatment but that their resistance to desiccation is very varied: diatoms appear to be entirely killed off and are therefore presumably all present in the soil in the vegetative condition; blue-green algae, except for one small sheathless species of *Oscillatoria* which was completely killed off, appear to be unaffected by the treatment, while the unicellular green forms reacted quite specifically. In one particular sample, *Pleurococcus vulgaris* Menegh, the most resistant species, was reduced only to rather more than one-third, while *Chlorococcum humicola* was reduced to one-twelfth of its numbers.

[1] Department of Bacteriology and Immunology.

In *Chlorella* sp. and *Heterococcus viridis* more than ninety-nine out of every hundred cells were killed off, and in *Bumilleria exilis* a few cells only survived. In fact, of a total algal population of about eighty thousand individuals per gram of soil, only about nine thousand survived after desiccation. This definite proof that the soil algae are actually present in large numbers in the vegetative condition raises their status as members of the soil population to a position not far short in importance of that of the other groups of soil organisms, the numbers of individuals in the Rothamsted soil being quite comparable with those of the protozoa."

Definite information that algae actually *multiply* in soil, however, has not been encountered by the author. It was to supply this missing link in the chain of evidence that algae lead an active life in normal soils that the following experiments were carried out.

EXPERIMENTAL

Samples of peat (Niedermoor), garden loam, and sandy field soil were taken from the field when in a moist but not wet condition. Unfortunately no moisture equivalent determinations were made but it may be stated that the soil was friable and appeared to contain free water to the extent of somewhere from one-half to two-thirds of the water holding capacity. This soil was weighed in 100-gm. amounts and added to 250-cc. Erlenmeyer flasks, which were then plugged with cotton wool and immersed in a water bath for 90 minutes at 85°C. They were removed and weighed and left until they had lost in weight about 10 gm. Heating the soil thus does not kill all the bacteria. It greatly reduces their numbers but the total numbers again rise to very high levels relatively soon. The fungi, algae, and protozoa are all, or nearly all, eliminated, and their bodies undergo a bacterial decomposition similar to what happens after moistening soil following air drying. Carbon dioxide and ammonia production increases and ammonia rather than nitrates, accumulates in the soil but it is well known that most algae are able to use nitrogen in either form.

At this time dilute suspensions of two pure cultures of unicellular algae[2] isolated from soil (11) were prepared, and 10 cc. of each were added to duplicate flasks of each of the three samples of soil, making 12 flasks in all. An equal number of flasks of soil were left uninoculated, and 10 cc. of sterile water was added. The flasks were all wrapped in heavy paper and placed in tin cans in which a few holes plugged with cotton wool, had been prepared for ventilation. All of the cans were placed in a dark cupboard and left for 6 and 8 months. Once a month sterile distilled water was added to make up for loss by evaporation. Tubes containing inorganic media inoculated with algae failed to show growth, indicating that the darkness was complete. After the long incubation the soil was removed, carefully mixed, and the numbers obtained by the dilution method using the tables of Halvorson (5) and the liquid medium of Bejerinck (11). At the time of inoculation the algae were counted

[2] The algae were identified as *Chlorella* sp. and as probably *Scenedesmus costulatus* Chodat var. *chlorelloides* Bristol Roach.

TABLE 1

Number of viable units of Chlorella *sp. per gram of partially sterilized soil at time of inoculation and after 6 months' incubation*

NUMBER OF POSITIVE TUBES PER DILUTION				MOST PROBABLE NUMBER OF VIABLE UNITS PER GRAM OF SOIL
1:10	1:100	1:1,000	1:10,000	
At inoculation				
4	0	0	..	3.8
2	1	0	..	
Sandy soil. After incubation				
10	8	1	..	125
10	7	0	..	
Peat. After incubation				
..	10	4	0	642
..	10	6	0	
Loam. After incubation				
..	10	9	2	2,280
..	10	9	2	

TABLE 2

Number of viable units of Scenedesmus costulatus *var.* chlorelloides *(?) per gram of partially sterilized soil at time of inoculation and after 8 months incubation*

NUMBER OF POSITIVE TUBES PER DILUTION				MOST PROBABLE NUMBER OF VIABLE UNITS PER GRAM OF SOIL
1:10	1:100	1:1,000	1:10,000	
At inoculation				
7	2	0	..	13.3
7	1	1	..	
Sandy soil. After incubation				
10	7	1	..	97.1
10	6	0	..	
Peat. After incubation				
10	10	3	..	565
10	10	5	..	
Loam. After incubation				
..	10	8	1	1,330
..	10	7	1	

in a similar manner in two flasks of soil prepared for the purpose. The first dilution was made by adding water to 10 gm. of moist soil to the 100-cc. mark. After that, 10 cc. of each dilution was added to 90 cc. of sterile inorganic medium, and 1 cc. of each decimal dilution added to Beijerinck's medium in replicates of 10 and incubated for 8 weeks in a suitable light. The estimates of numbers of viable algae were made in the usual manner by using Halvorson's tables, tubes which showed any green growth being counted as positive. None of the tubes inoculated with the soil to which no algae had been added showed growth. The results for the others are shown in tables 1 and 2.

SUMMARY

The results of the experiments show that algae inoculated in soil partially sterilized by heat are able to increase as much as 500 fold when incubated at room temperatures in total darkness for long periods of time. They offer added evidence that soil is a habitat for certain green algae and that the algae multiply in soil under conditions of darkness and of moderate moisture.

REFERENCES

(1) Bristol, B. Muriel 1920 On the alga flora of some desiccated English soils: an important factor in soil biology. *Ann. Bot.* 34: 35–80.

(2) Bristol Roach, B. Muriel 1927 On the algae of some normal English soils. *Jour. Agr. Sci.* 17: 563–588.

(3) Bristol Roach, B. Muriel 1928 The present position of our knowledge of the distribution and functions of algae in the soil. *Proc. First Internatl. Cong. Soil. Sci.* (Washington, 1927) 3: 30–38.

(4) Esmarch, Ferdinand 1914 Untersuchungen über die Verbreitung der Cyanophyceen auf und in verschiedenen Böden. *Hedwigia* 55: 224–273.

(5) Halvorson, H. O., and Ziegler, N. R. 1932 Application of statistics to problems in bacteriology: I. A means of determining bacterial populations by the dilution method. *Jour. Bact.* (In press).

(6) McLennan, E. 1928 The growth of fungi in soil. *Ann. Appl. Biol.* 15: 95–109.

(7) Moore, G. T., and Karrer, J. L. 1919. A subterranean alga flora. *Ann. Missouri Bot. Gard.* 6: 281–307.

(8) Moore, G. T., and Carter, Nellie. 1926 Further studies on the subterranean algal flora of the Missouri Botanical garden. *Ann. Missouri Bot. Gard.* 13: 101–140.

(9) Peterson, J. B. 1915 Studier over danske aerofile Alger. Mem. Acad. Roy. Sci. Lett. Danemark, Copenhague. 7. ser., Sect. d. Sci. 12, no. 7. (Original not seen.)

(10) Robbins, W. W. 1912. Algae in some Colorado soils. *Colo. Agr. Exp. Sta. Bul.* 184: 24–36.

(11) Skinner, C. E. 1932 Isolation of green algae in pure culture from soil by a simple technique. *Plant Physiology* (In press).

NOTES ON THE ASSOCIATION OF MICROÖRGANISMS AND ROOTS[1]

CHARLES THOM AND HARRY HUMFELD

United States Department of Agriculture

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Greater numbers of microörganisms in the immediate neighborhood of plant roots than outside the root-zone or rhizosphere have been reported by various workers. Some "influence" of the roots which brought about these increases was assumed, but not analyzed. The samples from which cultures were made were carefully collected as "representative" of the area to the depth designated, then homogenized, and sieved to insure the presence of of earthy materials only. Smith and Humfeld[2] before the Society of American Bacteriologists in December, 1929, reported experiments which pointed to such localization of bacteriological activities upon organic masses in soil as to lead to the recommendation that further studies be directed more to the determination of the extent and nature of these localized activities than to further attempts to define population levels by dilution cultures from aliquots drawn from homogenized samples representing great areas and vast masses of soil.

Starkey followed the earlier practices in parts I, II, and III (2, 4, 5) of his studies of the micropopulation of the rhizosphere, but has offered intensive studies of the numbers of microorganisms on the roots themselves in part IV (6). His survey of the whole literature of the field makes a general discussion of the historical aspects of the subject unnecessary.

In his recent paper (6) Starkey took the usual type of samples with elaborate care at three different distances from the main root of the plant; the outermost sample was planned to be clearly outside the zone of influence; the second was taken at an intermediate distance; the third close to, but not in contact with the main center of the root system. The studies included total colony counts on albumin agar and differential counts for fungi, actinomycetes, mucoid colonies, and radiobacter. The plants seclected included two series of three species each: (*a*) second-year growth of the biennials, table beets, mangel beets, and sweet clover, and (*b*) fresh plantings of bush beans, mangel beets, and corn. His fourth sample consisted of scrapings of the roots themselves, which necessarily included adherent soil particles. As in previous reports, the numbers of microörganisms were greater as the samples entered the root zones and approached the main roots of the plants but reached totals which averaged 45.3 times (6, p. 375) as many bacteria in all of the samples of root scraping as in all of the samples of nearby soil. In the legumes this ratio was 113.3, but in the non-legumes it fell to 11.3. These results indicate clearly that increased numbers of microörganisms in the root zone are due to organisms actually in or on the roots themselves.

Independently following the same general lines, several series of samples were studied in the laboratories of the Bureau of Chemistry and Soils during the past year, which give results in general harmony with those of Starkey and extend the field of investigation in certain directions.

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This paper was presented at the annual meeting of the American Society of Bacteriologists, at Baltimore, Maryland, December 30, 1931.

[2] Abs. in *Jour. Bact.* 19 (1): 43–44. 1930.

STUDIES OF RYE, VETCH, AND ALFALFA ROOTS

The procedure used in sampling for the comparative study of the populations in soil and on the roots of rye, vetch, and alfalfa during the early spring of 1931 follows. A typical healthy plant was dug up, with care to disturb the root system as little as possible. The adhering soil was shaken off and labelled "soil adjacent to roots." The fibrous roots were cut from the main root system for a separate sample. This included the soil particles which adhered so closely that vigorous shaking did not dislodge them. A sample was taken from fallow soil outside of the root zone.

TABLE 1

Distribution of microörganisms in relation to roots of plants in different soils

TYPE OF SOIL	KIND OF MATERIAL	CROP	pH	NUMBER OF FUNGI PER GRAM	NUMBER OF ACTINOMYCES PER GRAM	NUMBER IN TOTAL PLATE COUNT PER GRAM (BACTERIA)
Keyport clay loam	Soil	.....	5.7	130,000	4,030,000	30,000,000
	Soil adjacent to roots	Alfalfa	5.8	251,000	4,010,000	98,350,000
	Fibrous roots	Alfalfa	6.2	620,000	13,750,000	423,000,000
Keyport clay loam	Soil	.....	6.1	90,000	715,000	99,500,000
	Soil adjacent to roots	Rye	6.9	121,000	890,000	92,500,000
	Fibrous roots	Rye	7.2	575,000	2,600,000	506,000,000
Leonardtown clay loam	Soil	.....	6.1	287,000	2,500,000	24,050,000
	Soil adjacent to roots	Rye	6.8	307,000	1,250,000	47,050,000
	Fibrous roots	Rye	7.6	1,430,000	.....	527,500,000
Leonardtown clay loam	Soil	.....	6.8	261,000	1,325,000	42,050,000
	Soil adjacent to roots	Vetch	7.0	260,000	.....	162,500,000
	Fibrous roots	Vetch	7.0	1,340,000	1,550,000	495,000,000
Collington fine sandy loam	Soil	.....	6.4	22,000	1,700,000	18,000,000
	Soil adjacent to roots	Rye	6.4	32,000	1,950,000	21,650,000
	Fibrous roots	Rye	7.1	330,000	3,350,000	292,000,000

Ten grams of each sample were weighed out, triturated in a mortar, and suspended in 250 cc. of sterile water. Dilutions on the basis of 10 cc. of inoculum to 90 cc. of sterile water were used, and the series carried far enough to insure a suspension which would give a favorable number of colonies for counting. Five cubic centimeters of the suspension were placed in a sterile petri dish and the desired medium was added.

The media used [following Smith and Humfeld (2)] were acid dextrose soil extract agar for the fungi, glycerine nitrate soil extract agar for the actinomyces, and soil extract agar for the total plate counts of bacteria.

The plates for fungous colonies were incubated for 3 days, those for actinomyces 10 to 14 days, and the total count plates for 21 days.

Table 1 gives a summary of the results obtained in this first series of samples.

The soils were Keyport clay loam, Leonardtown clay loam, and Collington fine sandy loam. The pH of the soils varied from 5.7 to 6.8, and the pH of the roots was from 6.2 to 7.6. In each case the roots were somewhat less acid than the soil.

The number of fungi on the fibrous roots was considerably higher than the number in the soil. The actinomycetes in most cases show an increase on the roots. However, difficulty was experienced in obtaining an accurate count because fungi and bacterial spreaders grow rapidly on this medium and often mask or inhibit actinomyces so that an accurate count was not always possible. The total plate counts, which were mostly bacteria, were always very high from the fibrous root material, varying from 292 million for the rye roots from the sandy soil, to 527 million for the rye roots from the clay loam. The number in the soil was the usual number, varying from 18 million for the sandy soil to 99 million for the Keyport clay loam.

The number of microörganisms in the rhizosphere, that is, the soil adjacent to the roots, was usually higher than the number in the soil. outside the root zone.

CORN ROOTS IN SEVEN SOILS

In connection with another experiment in progress in the greenhouse, it was possible to obtain roots of corn which had been grown under identical conditions, except that the soils varied. The corn at time of sampling was about 8 weeks old and about 2 feet high. The plots had grown a number of successive crops of corn and each crop had received an application of phosphate and potash.

The soils were Caribou loam, pH 4.5; Collington fine sandy loam, pH 4.8; Keyport clay loam, pH 5.9; Carrington loam, pH 7.; Houston clay loam, pH 7.8; a silt loam from Colorado, pH 7.9; and an "alkali" soil from Colorado, pH 8.1. The same media were used for fungi, actinomycetes, and total plate counts as in the previous experiments. The results are summarized in table 2.

In the acid soils and in the neutral soil the roots were less acid than the soil; in the alkaline soils the roots were less alkaline than the soil. The colony counts of fungi, actinomycetes, and bacteria varied as in the previous series of samples. It was noted that the number of fungi on the roots of corn was greatest in the most acid and in the most alkaline soil. The number of actinomycetes did not show any increase and the number of bacteria was greater on the roots grown in soil slightly acid or neutral.

Group identification of the fungi showed that species of Trichoderma predominated in the roots in acid to neutral soils, whereas in the three alkaline soils biverticillate Penicillia (the *P. luteum* group) predominated.

TABLE 2

Distribution of microörganisms in relation to corn roots in soils of different acidity

TYPE OF SOIL	KIND OF MATERIAL	pH	NUMBER OF FUNGI PER GRAM	NUMBER OF ACTINOMYCETES PER GRAM	NUMBER OF TOTAL PLATE COUNTS (BACTERIA)
Caribou loam	Soil	4.5	145,000	425,000	24,500,000
	Soil adjacent to roots	4.8	230,000	.....	19,000,000
	Fibrous roots	6.1	4,100,000	.....	43,350,000
Collington fine sandy loam	Soil	4.8	100,000	1,100,000	5,500,000
	Soil adjacent to roots	5.2	800,000	.....	26,000,000
	Fibrous roots	5.6	7,000,000	.....	136,000,000
Keyport clay loam	Soil	5.9	105,000	465,000	33,300,000
	Soil adjacent to roots	6.1	200,000	.....	53,150,000
	Fibrous roots	6.7	555,000	1,900,000	260,000,000
Carrington loam	Soil	7.0	141,000	5,000,000	35,000,000
	Soil adjacent to roots	7.0	70,000	2,225,000	38,000,000
	Fibrous roots	7.6	700,000	5,000,000	270,000,000
Houston clay	Soil	7.8	155,000	955,000	25,450,000
	Soil adjacent to roots	8.0	.....	.....	.....
	Fibrous roots	7.5	300,000	1,000,000	82,000,000
Silt loam Colorado	Soil	7.9	205,000	1,020,000	41,000,000
	Soil adjacent to roots	7.7	162,000	490,000	48,500,000
	Fibrous roots	7.5	352,000	1,400,000	60,000,000
"Alkali" soil Colorado	Soil	8.1	100,000	930,000	17,750,000
	Soil adjacent to roots	8.1	185,000	495,000	25,650,000
	Fibrous roots	7.5	1,315,000	450,000	138,000,000

EFFECT OF TOBACCO ROOT ROT ON THE NUMBERS OF MICROÖRGANISMS

As plants of tobacco, both healthy and infected with the tobacco root rot (Thielavia) were available, it was thought that some interesting information might be gained by plating out the organisms on the roots of these plants. Three different samples were obtained: (*a*) from a highly resistant Burley strain grown on badly infected soil; (*b*) from a susceptible strain grown on soil free from infection as far as known; (*c*) from a susceptible strain grown on badly infected soil, in fact, grown adjacent to the resistant strain.

The results obtained are shown in table 3. It will be seen at a glance that large differences exist. Total plate counts gave 136 millions on healthy, uninfected plants grown on root-rot free soil, 223 millions on the resistant strain in infected soil and 1,620 millions on the susceptible strain on infected soil. The results on the other media were proportionate in a general way. It was found that radiobacter was practically absent on the roots of plants in

TABLE 3

Numbers of microörganisms on washed roots on tobacco plated on different media

KIND OF ORGANISMS	MEDIUM	FIBROUS ROOTS RESISTANT—BURLEY VARIETY ON INFECTED SOIL	LARGER ROOTS RESISTANT—BURLEY VARIETY ON INFECTED SOIL	FIBROUS ROOTS FROM UNINFECTED SOIL	LARGER ROOTS ON UNINFECTED SOIL	FIBROUS ROOTS BADLY INFECTED	NUMBERS ON LARGER ROOTS
Total colony count	Soil extract agar	233,000,000	69,000,000	136,000,000	27,000,000	1,620,000,000	181,000,000
Fungi	Acid dextrose nitrate agar	45,000	20,000	62,000	142,000	182,000	20,000
Actinomyces	Glycerine nitrate soil extract agar	4,500,000	2,500,000	2,300,000	550,000	3,200,000	1,000,000
Mucoid colony count	Glycerine nitrate soil extract agar	3,000,000	3,700,000	1,800,000	.....	35,000,000	4,700,000
Total colony count	Glycerine nitrate soil extract agar	115,000,000	74,000,000	40,000,000	13,200,000	1,500,000,000	102,000,000
Mucoid colony count	Crystal violet glycerine nitrate soil extract agar	850,000	600,000	140,000	250,000	50,000,000	200,000
Total colony count	Crystal violet glycerine nitrate soil extract agar	41,750,000	55,000,000	1,140,000	625,000	154,000,000	4,400,000
Mucoid colony count	Mannite agar	5,800,000	.....	1,000,000	500,000	50,000,000	4,000,000
Total colony count	Mannite agar	212,000,000	125,000,000	29,600,000	7,100,000	1,400,000,000	112,000,000
Total colony count	Albumin agar	245,000,000	200,000,000	50,700,000	17,800,000	640,000,000	105,000,000
Total colony count	Dextrose agar	78,000,000	40,700,000	19,600,000	20,000,000	865,000,000	130,000,000
Total colony count	Starch agar	225,000,000	168,000,000	37,500,000	20,500,000	1,620,000,000	122,000,000
Total colony count	Peptone agar	230,000,000	135,000,000	10,500,000	20,000,000	890,000,000	165,000,000

uninfected soil, whereas 50 million per gram were present on the susceptible infected roots. The total number of colonies on mannite agar and on starch agar was especially high. No doubt, the root-rot organism weakened the resistance of the root cells against invasion by other organisms and as the root cells died the number of microörganisms present increased tremendously.

In the preliminary experiments reported in table 1, three soils were studied: Keyport clay loam with pH at 5.7 and 6.1, Leonardtown clay loam at pH 6.1 and 6.8, and Collington fine sandy loam at pH 6.4; three crops, rye, vetch, and alfalfa were grown upon them. In each case, the root samples with such soil particles as adhered to them were somewhat less acid than the soil in which they had grown. Of the colony counts of microörganisms the actinomycetes showed less differences than the bacteria or fungi. Their numbers from the roots varied from slight increases or decreases to about 4 times those found in the surrounding soil; the fungous colonies were 5 to 15 times as numerous on the roots, and the bacteria 5 to 20 times as numerous on the roots as in the soil. Although the procedures were so different that the figures are not directly comparable, these results may be regarded as fully in harmony with those of Starkey (6).

These results suggested the utilization of the corn already growing in the greenhouse upon seven plots of soil differing in origin, texture, and acidity but under comparable conditions of temperature, illumination, and watering. These plots varied from fine sandy loam to heavy clay and from pH 4.5 to pH 8.1. Here again, with a single crop, the root samples from the corn grown upon the acid soils were less acid than the soil and those grown upon alkaline soils were less alkaline. There was a clear tendency of the root systems to maintain a reaction approaching the neutrality zone between pH 6 and pH 7.5 rather than to show the acidity or alkalinity of the soil.

The microörganisms outside of the root tissues inhabit a sort of horizon zone between the root juices with one reaction and the contrasting solution of the surrounding soil. Comparison of the colony counts of fungi shows 4,100,000 to the gram on the roots from Caribou at pH 4.5; 7,000,000 from Collington, a loose textured fine sandy loam, at pH 4.8; 700,000 from Carrington of texture similar to Caribou, but at pH 7.0, and up to 1,315,000 again from roots grown in the "alkali" sample from Colorado at pH 8.1, which also is a fairly open textured soil. The high figures for bacteria were from roots grown in Keyport at pH 6.7 which gave 260,000,000, and in Carrington (pH 7.0), 270,000,000 to the gram of roots. These figures harmonize in a general way with the experience that fungous colonies are reduced where conditions are favorable to excessive growth of bacteria and vice versa, but the data are inadequate more than to show the trend.

The figures already discussed showed so definite a relation between the roots and the microörganisms that another series of cultures was made from healthy and infected tobacco roots. For this purpose tobacco plants already full grown under these contrasting conditions were available. Tobacco roots

present the opportunity to obtain samples of fibrous roots and scrapings from large roots. The three samples show the conditions produced about resistant roots in infected soil, susceptible roots in uninfected soil, and badly infected roots.

The total colony counts on soil extract agar were 27 millions per gram on healthy roots in uninfected soil; 233 millions on roots of a variety of tobacco resistant to the disease in infected soil; and 1,600 millions on the roots of tobacco of a variety not resistant in infected soil. The detailed figures in table 3 give some picture of the great numbers of organisms and the varieties of activity presented in connection with root diseases.

Colony counts of molds such as these reported here have a purely arbitrary value. Spores scarcely occur in the deeper areas about the root system. The colonies found represent the fragments of mycelium resulting from the grinding of the sample. When that grinding is all done by the same person with a reasonably standardized procedure, the results at least indicate the relative activities of the species viable on the media used. It is interesting to find that corn roots on acid soil are accompanied by abundant growth of Trichoderma which is largely absent on the sample from alkaline soil but replaced by the "biverticillate" Penicillia.

In addition to the cultures, microscopical studies of fresh roots were made for supplementary information especially as to the molds and their distribution. Mold hyphae are usually difficult to find in mounts made directly from earthy masses, as noted by Conn in his studies of microscopic counting methods, and by others. Upon and in fibrous roots, however, mold hyphae are nearly always evident. For example, one rootlet 200μ in diameter showed five large adherent brown hyphae of a single type of mold almost equally spaced for a considerable distance. These hyphae showed various anastomoses between those on the side of the rootlet seen with the microscope. Other fungous hyphae were present in the same field. On other rootlets penetration of superficial cells by mold hyphae was seen. Among root hairs, the actual invasion of the lumen of the hair by hyphae was not uncommon. As these are not isolated observations, it is concluded that mold growth microscopically seen in contact with and often inside the cells of the root cortex is common enough to justify the colony counts observed in culture.

Incidentally, myriads of bacteria appeared in the microscopic fields examined for molds although there was no further attempt to follow their distribution with the microscope.

SUMMARY

The authors report results in harmony with those of Starkey in indicating that (*a*) there are increased numbers of microörganisms in samples of soil taken in proximity to plant roots; (*b*) that these numbers are many times greater when the roots themselves are examined.

The soil samples studied showed acidities expressed as pH 4.8 to pH 18. and from clay loam to sandy loam.

The samples of fibrous roots with adherent soil particles gave reactions less acid than the acid soil and less alkaline than the alkaline soils, the figures ranged from pH 5.6 in one sample of roots from Collington loam at pH 4.8 to roots at pH 7.6 on Carrington loam at pH 7.0 on the one side; and on the other, roots at pH 7.5 on an "alkali" soil from Colorado testing pH 8.1. The high bacterial counts obtained are characteristic of situations which approach the zone around neutrality, i.e., pH 6 to 7.5.

Mold cultures from corn roots in acid soil produced colonies predominantly Trichoderma; corn roots from alkaline soil seemed to be associated predominantly with Penicillia of the biverticillate series (*P. luteum* and its allies).

High colony counts of fungi occurred on the very acid soils and the very alkaline samples.

The data presented justify the conclusion that corn roots penetrating through masses of soil of either strongly acid or strongly alkaline reaction, still maintain their own reaction in or near the general zone between pH 6 and pH 7.5 in which bacteriological activities are much more intense than under more acid or alkaline conditions. In doing this they create about them a very narrow zone favorable to bacterial and mold activity.

Greatly increased numbers were shown upon samples of roots known to be infected by root rot or to have grown in heavily infected soil.

REFERENCES

(1) SMITH, N. R. 1928 The identification of B. radiobacter and its occurrence in soil. *Jour. Bact.* 15: 20–21.

(2) SMITH, N. R., AND HUMFELD, H. 1930 Effect of rye and vetch green manures on the microflora, nitrates, and hydrogen-ion concentration of two acid and neutralized soils. *Jour. Agr. Res.* 41: 97–123.

(3) STARKEY, R. L. 1929 Some influences of the development of higher plants upon the microörganisms in the soil: I. Historical and introductory. *Soil Sci.* 27: 319–334.

(4) STARKEY, R. L. 1929 Some influences of the development of higher plants upon the microörganisms in the soil: II. Influence of the stage of plant growth upon some of the abundance of the organisms. *Soil Sci.* 27: 355–378.

(5) STARKEY, R. L. 1929 Some influences of the development of the higher plants upon the microörganisms in the soil: III. Influence of the stage of plant growth upon some of the activities of the organisms. *Soil Sci.* 27: 433–444.

(6) STARKEY, R. L. 1931 Some influences of the development of higher plants upon the microörganisms in the soil: IV. Influence of the proximity to roots on abundance and activity of microörganisms. *Soil Sci.* 32: 367–393.

INFLUENCE OF CONTINUOUS AERATION UPON THE GROWTH OF TOMATO PLANTS IN SOLUTION CULTURES[1]

H. E. CLARK AND J. W. SHIVE

New Jersey Agricultural Experimental Station

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In the course of an experiment in which it was necessary to grow tomato plants in solution cultures it was observed that the roots of the plants did not grow down through the solution to the bottom of the culture jars. Instead, the roots were greatly branched and thickly packed at the surface of the solution in the necks of the jars. Since it was desirable to prevent this packing of the roots in the jars, and since the crowding at the surface of the solution suggested a lack of oxygen in the solution, the following experiment was carried out to show the effect of vigorous and continuous aeration of the culture solution upon the growth of tomato plants.

No attempt was made to study the oxygen content of the solution. Allison and Shive (2) have previously shown that aeration of solution cultures which maintained the maximum supply of dissolved oxygen in the culture solutions renewed intermittently had no apparent influence upon the growth of soybean tops, but it produced considerable increase in root development. When the solution was continuously renewed, however, they found that aeration produced a marked increase in the growth of both tops and roots of soybeans.

CULTURE METHODS

Tomato seedlings of the Marglobe variety were germinated in sand and were transferred to the culture solution when they were about 6 days old. The seedlings were suspended in cork stoppers in the 2-quart fruit jars used as culture vessels, in a manner similar to that described by Tottingham (5). Three plants were used in each culture vessel. Plants grown during an experimental period of 81 days were started in the 2-quart jars and single plants were removed from these at the age of 27 days. These plants were individually placed in gallon candy jars shown in plate 1, figure 2, in which they were grown for the remainder of the experimental period. Large plants with extensive root systems could be grown in these vessels. Both the 2-quart and the 4-quart culture vessels were covered with cardboard jackets to prevent the entrance of light.

A modified Tottingham solution, $T_3R_1C_3$, selected from those described by

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

Jones and Shive (3), was used for all cultures. The total osmotic concentrations of this solution was one atmosphere, and the partial volume-molecular concentrations of each of the four salts included were: KH_2PO_4 — .00633 *M*; $Ca(NO_3)_2$ — .00438 *M*; $MgSO_4$ — .00711 *M*; and $(NH_4)_2SO_4$ — .0014 *M*. This solution was one which had produced excellent growth of tomatoes in previous experiments. Supplements of boron in the form of boric acid and of manganese in the form of manganese sulfate were added to the culture solution in quantities sufficient to give a concentration of 0.5 p.p.m. of these elements. Freshly prepared ferrous sulfate in solution form was regularly added to the solutions at the rate of 0.5 p.p.m.

The solutions were renewed several times while the plants were still very small, but after 10 days, continuous renewal of the solutions was begun, the method used being that devised by Shive and Stahl (4). The rate of renewal while the plants were small was 1 liter every 24 hours.

At the same time that continuous renewal of the solutions was started, continuous aeration of one-half of the culture solutions was begun. The method of aeration was similar to that described by Allison (1), four or five bubbles of air per second escaping from the tube at the bottom of each culture jar. This aeration proceeded continuously until the time of harvest in all those cultures designated as "aerated." The cultures designated as "non-aerated" were not totally restricted to the oxygen normally dissolved in the solution, since the method of renewal employed (4) provides a small bubble of air for every drop of solution falling into the culture jar.

The plants in series A were placed in the culture vessels on July 13, 1930, three plants in each culture. They were harvested on August 25, and the green and dry weights of tops and roots were obtained in the usual way.

Series B and C were grown at a different season in 1931. The seedlings of series B were placed in the culture solutions on March 14 and were grown for 48 days, when they were harvested. The cultures of series C were also begun on March 14, but they were not harvested until they had been grown for 81 days. On account of the high rate of transpiration the rate of continuous solution renewal was increased on April 12 from 1 liter to 2 liters daily. On April 24 the osmotic concentration of the solutions of series C was reduced from 1 atmosphere to 0.5 atmosphere. Plate 1 shows photographs of plants 48 days old.

EXPERIMENTAL RESULTS

The data given in table 1 represent the average yields of plants grown in aerated and non-aerated solutions. These data clearly show the beneficial effect which aeration of the culture solution exerted upon the growth of both tops and roots of the plants. It will be observed that in each of the three series the average total dry weight yields of the cultures grown in aerated solutions were more than 50 per cent higher than the corresponding yields of those grown in the non-aerated solutions. The superior development of both tops and roots of the aerated plants is also clearly shown in plate 1.

This comparison between the cultures grown in the aerated and those grown in the non-aerated solutions is emphasized and quantitatively expressed by the data presented in table 2. These data represent the ratios of dry weight yields of the aerated to those of the non-aerated cultures grown under otherwise identical conditions. The markedly superior growth of both the tops and the roots of the aerated cultures is indicated by the high values of these ratios. The extremely high ratio of top growth given for the 48-day plants in series B (plate 1, fig. 1) is probably both an indication of the marked benefit from

TABLE 1

Green and dry weights of tomato plants grown in aerated and non-aerated culture solutions

CULTURES	AVERAGE WEIGHT PER PLANT			
	Green weight of tops	Dry weight of tops	Dry weight of roots	Total dry weight
	gm.	*gm.*	*gm.*	*gm.*
Series A, 48 days:				
Non-aerated	22.4	1.8	0.2	2.0
Aerated	41.0	3.0	0.3	3.3
Series B, 48 days:				
Non-aerated	45.5	5.8	0.9	6.7
Aerated	157.7	16.0	1.7	17.7
Series C, 81 days:				
Non-aerated	280.1	34.0	4.8	38.8
Aerated	537.3	63.1	7.4	70.5

TABLE 2

Ratios of dry weights of tops and roots and top/root ratios of tomato plants grown in aerated and non-aerated culture solutions

CULTURES	DRY WEIGHT RATIOS—AERATED/NON-AERATED CULTURES			DRY WEIGHT RATIOS—TOPS/ROOTS	
	Tops	Roots	Total	Non-aerated	Aerated
Series A, 48 days	1.67	1.50	1.65	9.0	10.0
Series B, 48 days	2.76	1.89	2.64	6.4	9.4
Series C, 81 days	1.86	1.54	1.82	7.1	8.5

aeration in crowded cultures and of the retarding effect upon growth of a deficient oxygen supply when containers are too small to accommodate adequately vigorous root systems. Three plants were included in each one of these cultures and the external environment at this season of the year was such as to promote excellent growth under these conditions. Lack of aeration of the culture solution, and in consequence an insufficient oxygen supply, is a very serious hindrance to normal growth and development and a potent factor in rendering worthless experimental data obtained from solution culture investigations.

The ratios of tops to roots given in table 2 emphasize a conclusion which is already suggested by the data in columns 1 and 2 of this table. It will be observed that these ratios of average dry weights in each of the three series are considerably higher for the aerated than they are for the corresponding non-aerated cultures, thus indicating that the influence of culture solution aeration upon top growth is relatively more pronounced than it is upon root growth. This relates, of course, to the tomato plant grown under the particular set of experimental conditions here specified.

Although the plants in the non-aerated solutions were much smaller, they started to blossom and to fruit earlier than did those in the aerated cultures. For this reason, the average total green weight of the fruits on plants of series C grown in the non-aerated solutions was somewhat greater at the time when harvested than that of the plants grown in the aerated solutions, the yield values being 399.7 and 321.0 gm. respectively. The weight of the fruit given does not represent the weight of fruit produced by a fully matured plant, of course, but simply shows the weight of the immature fruit already produced at this particular stage of development. However, at the time of harvest (81 days) the aerated plants showed every evidence of yielding a much larger crop of fruit than the non-aerated plants could yield.

REFERENCES

(1) ALLISON, R. V. 1922 The relation of aeration to the development of the soybean plant in artificial culture. *N. J. Agr. Exp. Sta. Am. Rpt.* 1921: 338–344.

(2) ALLISON, R. V., AND SHIVE, J. W. 1923 Studies on the relation of aeration and continuous renewal of nutrient solution to the growth of soybeans in artificial culture. *Amer. Jour. Bot.* 10: 554–566.

(3) JONES, L. H., AND SHIVE, J. W. 1921 Effect of ammonium sulfate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulfate as sources of iron. *Jour. Agr. Res.* 21: 701–728.

(4) SHIVE, J. W., AND STAHL, A. L. 1927 Constant rates of continuous solution renewal for plants in water cultures. *Bot. Gaz.* 84: 317–323.

(5) TOTTINGHAM, W. E. 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* 1: 133–245.

PLATE 1

INFLUENCE OF AERATION UPON ROOT AND TOP GROWTH OF TOMATOES IN SOLUTION CULTURES

FIG. 1. TYPICAL CULTURES FROM SERIES B
Left, aerated; right, non-aerated

FIG. 2. TYPICAL CULTURES FROM SERIES C
Left, aerated; right, non-aerated

CONTRIBUTION TO OUR KNOWLEDGE OF THE CHEMICAL NATURE AND ORIGIN OF HUMUS: I. ON THE SYNTHESIS OF THE "HUMUS NUCLEUS"[1]

SELMAN A. WAKSMAN AND K. R. N. IYER

New Jersey Agricultural Experiment Station

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The subject of the chemistry of humus, or the dark colored organic matter in soils, peats, and composts, has attracted considerable attention during the last 150 years. Although chemists and agronomists have frequently used the terms "humus," "humic acid," "ulmic acid," "humin," "ulmin," and numerous others to designate complexes with seemingly different chemical properties, it has become now generally recognized that all these preparations were not definite compounds but depended largely upon the procedures used for their extraction. All these dark colored organic complexes are left in the soil or are formed there as a result of the decomposition of plant and animal residues. The nature of the soil, the nature of the residues, and the conditions of decomposition largely influence their chemical nature.

Numerous attempts have also been made since 1830 to synthesize by chemical procedures dark colored organic complexes which would have the same properties as the humus of the soil, of peat, and of decomposing plant residues (23). The dark color of the soil humus, its solubility in dilute alkalies, and its precipitation by acids, as well as its relatively high carbon content, served as a basis for these attempted syntheses; numerous complexes were thus prepared having properties similar to those of the humus in soils and composts. The treatments consisted in the action of acids at high temperatures on carbohydrates, the interaction of sugars with amino acids, and the oxidation of phenol and its derivatives. The dark colored preparations formed under these conditions cannot be compared with full justification with those formed under natural conditions. The natural products are markedly different, both because of the difference in the initial substances from which the humus is formed and because of the nature of the processes whereby this is brought about. There are also marked chemical differences which are not always taken into consideration, such as the relatively constant nitrogen content of the natural preparations. All the attempts to synthesize humus by chemical agencies may be considered to have failed so far in giving preparations which would be identical in all respects with the humus formed under natural conditions; the information thus accumulated may be considered to have had no influence

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

upon our knowledge of the chemical nature of humus in soils and composts. Actually the syntheses tended to confuse the subject rather than to clarify it and explain the processes governing the formation of humus, its nature, and its decomposition.

This failure to synthesize complexes comparable to soil humus is due largely to the fact that, in all the attempts to obtain dark colored substances by treatment of organic complexes with chemical reagents, no consideration was given to the natural processes which are responsible for the formation of humus from plant residues, namely, the action of microörganisms. Although these investigations were begun at a time when microbiology, especially the microbiology of the soil, was non-existent, they have been considered in the same manner during the last 50 years, which witnessed an extensive development of our knowledge of the rôle of microörganisms in soil processes. As a result of this, a century of progress of organic chemistry has contributed little to the knowledge of the chemical composition of the dark colored organic complexes of the soil. To be sure, considerable information has accumulated concerning the physico-chemical properties of humus (17); it has also been shown that humus is not so simple in chemical composition as originally assumed and that numerous substances can be isolated from it (19). These were the only positive phases of humus investigation, outside, of course, of the accumulated knowledge concerning the rôle of humus in soil processes and in the growth of plants and microörganisms. However, the fundamental problem still remained unsolved, namely, the origin and chemical nature of the basic complex which makes up the soil humus. One would hardly be justified in considering the continuously increasing number of "humic acids," which were in most instances labels for different preparations rather than for definite chemical entities, as contributing to the advance of our knowledge of the soil humus. Here the ideas have not progressed much from those in vogue a century ago, with a mere substitution of new names for old ones.

With the advance of our knowledge of the chemical composition of plant and animal residues, with the growing appreciation of the biochemical processes carried out by soil microörganisms and with the recognition of the importance of these microörganisms in the decomposition of plant and animal residues, especially when it was found that not all the chemical constituents of those residues decompose at a uniform rate, the chemical nature of humus and the processes leading to its formation have become better understood. Of special importance in this connection has been the recognition of the fact that, among the plant constituents, the lignins are more resistant than the other organic complexes to decomposition by fungi and bacteria, which are responsible for the transformation of plant residues in soil, peat, and composts. The accumulated information regarding the large quantities of proteins and other organic nitrogenous complexes which are synthesized during the decomposition of the plant residues, has further contributed to our knowledge of the nature and formation of humus.

These two complexes, namely, the lignins and the proteins, make up as much as 70 to 80 per cent of the humus in mineral soils (25, 29); in lowmoor, forest, and sedimentary peats, as well as in composts which have undergone considerable decomposition. The increase in these two chemical constituents of the humus accounts for its high carbon and nitrogen content. This is due primarily to the high carbon content of the lignin and to the high nitrogen content of the protein. Further, the fact that a definite carbon-nitrogen ratio, approaching 10 to 1, has been found for the organic matter or humus of many mineral soils points to the possibility of the existence of a certain chemical relationship between the lignin and the protein groups. The fact, however, that this carbon-nitrogen ratio is frequently modified, as by increasing depth of soil, by varying climatic conditions, and by the nature of the plant residues and their decomposition, tends to show that if such chemical relations exist they are not absolute but are variously modified.

One must also emphasize the fact that, in addition to the lignin-like complexes (or lignin derivatives) and the protein-like complexes, humus contains also a number of other organic substances, most important of which are certain hemicelluloses, largely uronides, certain fats and waxes, higher alcohols, and organic acids. The nature and abundance of these compounds depend upon the nature of the plant substances which contribute to the formation of the humus, the extent of their decomposition, the soil reaction, and climate.

Various attempts have recently been made to separate the organic matter of the soil (20) into "humus" and "non-humus" fractions, or into "humic" and "non-humic" bodies (22, 9), by the use of different reagents, such as acetyl bromide (12, 21) or hydrogen peroxide (15). Such separations, however, are not based upon a better knowledge of the chemical nature and origin of the soil organic matter than that done by the earlier investigators, who have separated this organic matter into "humins" and "humic acids," on the basis of their solubility in alkalies (2, 17). These procedures further emphasized the fact that the soil organic matter, to which alone the term "humus" can be justly applied, can be separated into alkali-soluble and alkali-insoluble complexes; the latter can be, however, further dissolved, partly at least, in hot alkali solutions. The alkali-soluble part gives, on neutralization with acids, a precipitate, while certain substances are left in solution. It is this alkali-soluble portion, after it has been precipitated by acids, which has attracted the greatest attention, since it forms a large part (30 to 60 per cent) of the humus in soils and in peats. It is also this fraction which seems to be more resistant to further decomposition than the other humus constituents. It is this fraction which resembles in its properties lignin, and to which the terms "humus" and "humified portion" have frequently been attached. Several theories have been recently suggested (5, 23) in which lignin was considered to be the mother substance of "humus" or the alkali-soluble and acid-precipitated part of humus.

The fact that lignin and other plant constituents are largely soluble in acetyl-bromide, while a part of the humus is insoluble, merely proves that in

the decomposition of the plant residues, certain substances are formed, either by the transformation of some of the original plant constituents or through processes of synthesis by microörganisms, which are not acted upon by this reagent.

Although it is usually stated that humus is highly resistant to decomposition, actually it decomposes very slowly, otherwise its accumulation in the soil would increase indefinitely; under conditions of continuous cultivation, the humus content of the soil is gradually diminished. Various microörganisms, especially certain fungi, are known to be capable of decomposing lignins; there is no reason, therefore, to assume that these organisms should not be able to decompose as well the complexes which may be formed from lignin, either as a result of its transformation or combination with other plant, animal, or microbial constituents. In view of the resistance of the proteins in the humus to rapid decomposition, it was suggested that possibly a lignin-protein complex is formed which renders the proteins less readily subjected to attack by microörganisms.

Dehérain (3) observed the fact that the humus of manure is only moderately acted upon by microörganisms, liberating the nitrogen very slowly, in the form of nitrate; he suggested that this humus may be a mixture of lignin and protein, the latter being synthesized by bacteria. In view of the fact, however, that ordinary plant and animal proteins are readily attacked by bacteria, one could not be dealing here with an ordinary mechanical mixture of lignins and proteins, but with chemical compounds. Another suggestion was that the organic nitrogenous complexes in the humus are of a different chemical nature than common plant and animal proteins (1, 11). Lathrop (13) found previously that whatever the chemical nature of the proteins added to the soil, as shown by the distribution of amino groups, their decomposition leads to the formation of a new protein complex.

According to Hobson (10), the "humic acid" complexes of the soil may be regarded as adsorption compounds between lignins and proteins; the latter are thereby protected against bacterial attack. Alkali solutions of lignin and albumin were mixed and precipitated together by an acid; the amino nitrogen distribution (by the Van Slyke procedure) in this complex was found to be identical to that of the "humic acid" isolated from the soil. Jensen (11) also mixed alkali solutions of lignin and casein and precipitated the mixture with hydrochloric acid; the preparations, containing 3.9–4.1 per cent of nitrogen, were added to nutrient solutions containing the necessary minerals. These were inoculated with soil suspensions and incubated at 25°C. for 3 weeks; only traces of ammonia were formed in these solutions, but the control solution, containing a corresponding amount of nitrogen as casein, gave a transformation of 75–80 per cent of the nitrogen to ammonia. However, in the soil the resistance of this compound of lignin and casein to microbial attack was not so marked, and after 45 days only an insignificant part of the nitrogen was recovered as humus. Jensen concluded that lignin does not seem to be able to form such resistant adsorption compounds with every protein.

Demolon and Brigando (4) found that arable soils are able to fix dissolved proteins brought into contact with these soils; the protein thus fixed by the humus colloids could be nitrified in the soil just as readily as the pure proteins. The humic nitrogen, however, was not nitrified, thus leading to the conclusion that the stability of humic nitrogen in the soil is due to its specific chemical composition.

The rôle of tannins in rendering the organic nitrogenous compounds in humus more resistant to decomposition has also been suggested. Wollny (33) found that the decomposition of protein-rich soybean leaves is markedly delayed, as measured by the CO_2 evolution, when the material is moistened with a tannin solution; this was explained as due to the formation of a resistant tannin-protein complex. Moeller (16) considered the interaction of tannins with proteins as leading to the formation of "humus bodies." The gradual disappearance of the tannins in the process of decomposition of wood was explained by the formation of "humic acids," whereby the tannin thus combined no longer gives the tannin reaction and cannot be extracted with water. Tannin was found (14) to precipitate completely egg albumen, casein, milk protein, and other proteins; the ratio between the tannin and the nitrogen in the form of protein was about 20:1. Weis (30) has shown previously that albumoses and peptone are also completely precipitated by tannin. The problem remained to determine whether lignin and protein also form similar complexes.

The average chemical composition of the organic matter of six soils (25, 29) gave 43.27 per cent lignin ("lignin-humus complex" or "soil lignin"), 33.81 per cent protein or organic nitrogenous complexes, 11.02 per cent water-insoluble carbohydrates (cellulose, hemicelluloses, etc.), and 2.77 per cent ether and alcohol-soluble substances, thus accounting for 90.87 per cent of the total organic matter of the soil. Of this organic matter accounted for, 78.08 per cent was made up of two complexes, namely, the protein and the lignin. The same is true of lowmoor and sedimentary peats (26) and of well-decomposed manure (27). We are justified in concluding that the humus of mineral soils, of well-decomposed peats, and of composts consists predominantly of two chemical complexes, namely, lignins and proteins, with an admixture of other substances, such as fats and waxes, cellulose and hemicelluloses, and organic acids and alcohols; the nature and abundance of these accompanying substances depend upon the nature of the plant and animal residues added to the soil or used in making the compost, upon the extent of their decomposition, and upon the conditions under which this decomposition is taking place, such as reaction, moisture content, aeration, and temperature. Whether the two major complexes, namely, the lignins and the proteins, form one chemical complex in the humus, or whether they exist independently still remains to be determined.

The chemistry of humus thus resolves itself into a knowledge of the chemistry of two groups of substances, one group comprising the lignin and protein complexes, on the one hand, and another group comprising the remaining

organic complexes, on the other. This may be looked upon as a more modern paraphrase of the earlier conception of "humic acids," on the one hand, and of "humin" and "crenic" or "fulvic acids," on the other; or of the more recent conception of "humic substances," on the one hand, and of "non-humic compounds," on the other. This idea thus has a true historical background.

The subject of this contribution is to show that the humus of the soil consists of a major complex, which may be called, for want of a better name, "humus-nucleus" (28), consisting of the lignin and protein fractions, having certain chemical affinities; this complex is accompanied in the soil humus by other substances, either left from the decomposition of the plant and animal residues, or synthesized through the activities of the microörganisms. This complex was found to possess all the biological, chemical, and physical properties of the major fraction of the humus of the soil and is largely equivalent to the "humic acids" or "humic bodies." The term "humus nucleus" is not the happiest one, since the authors do not mean thereby a nucleus, in the grammatical sense, but merely the major fraction of humus in soils, peats, and composts. The term "synthetic humus" would have been more applicable, but since the latter has been so often applied, since the time of Malaguti, to dark colored substances formed by the treatment of carbohydrates with acids and with other chemical reagents, processes which have no equivalent under natural soil conditions, it is purposely avoided.

In carrying out these experiments, an attempt was always made to avoid as much as possible the use of high temperatures or of excessive treatment of the materials with chemical reagents. The idea was to establish: 1. what effect the presence of lignin has upon the decomposition of proteins and protein derivatives by microörganisms; 2. whether a stable (chemical ?) complex is formed between lignin and protein; 3. how this complex compares in the rapidity and nature of decomposition with similar complexes found in soils and in peats; 4. the comparative chemical and physico-chemical behavior of the two constituents; 5. what influence the synthesized complex has upon various important soil processes carried out by microörganisms.

EXPERIMENTAL

Preparation of lignin and proteins

Lignin has been defined by Wilstätter (31) as the unsaccharified part of the plant membrane. According to Wislicenus (32), lignin is "the sum of highly molecular, colloidally soluble bodies which are absorbed on the surface of cellulose fibers from the plant sap or cambium sap." These two definitions, to which numerous others could be added, are sufficient to illustrate the vagueness still existing concerning the nature of this important plant constituent. Chemically, lignin is considered by most chemists as a polymer of a complex which consists of a benzol ring, with several hydroxyl (OH), methoxyl (OCH_3), and carboxyl (COOH) groups. It was at first assumed that the methoxyl content of lignin is constant and lignin was frequently measured

quantitatively in plant residues and in soil by the amount of methoxyl present; more recent investigations, however, have shown that not only does the methoxyl content of lignin vary in different plant materials and even in different parts of the same material (18), but that in the decomposition of lignin by microörganisms, the methoxyl may be destroyed more rapidly than the rest of the molecule. A detailed study of the chemistry of lignin is found in the work of Fuchs (7), Freudenberg (6), and others. Attention should be called here to one characteristic property of lignin, namely, its solubility in alkali solutions, and the formation of definite chemical or adsorption complexes with bases. Proteins are amphoteric electrolytes and can combine with both acids and bases.

For the preparation of lignin from plant materials, a number of methods are at present available. There is no doubt that lignin obtained by different procedures varies in its chemical composition. The two methods most commonly employed are the following:

1. The treatment of the plant material in the cold with concentrated acids, such as 42 per cent hydrochloric or 66 to 80 per cent sulfuric; by this treatment the cellulose and other carbohydrates are brought into solution, and the lignin is left free as "acid lignin;" if the plant material is low in protein and in ash, as in the case of wood or straw, and the fats and waxes are removed by preliminary treatment with ether, a fairly uniform product is obtained. 2. The extraction of the plant material with sodium or potassium hydroxide solution, at a high temperature, thereby bringing the lignin into solution; on acidifying the alkali solution, the lignin is precipitated; if the precipitate is now boiled with an excess of the dilute acid, those hemicelluloses which were removed with the lignin will be hydrolyzed and brought into solution; the precipitate is filtered off and washed, giving fairly pure lignin, which is referred to as "alkali-lignin."

The yields obtained by the two procedures differ considerably, the amount of acid lignin obtained from the same plant material being considerably greater than that of alkali lignin.

For the following experiments, alkali lignin from wheat straw was prepared by treating the straw first with hot water and with hot dilute hydrochloric acid to remove the water-soluble substances, the starches, and a large part of the hemicelluloses and proteins. The residual material was then extracted with aqueous 4 per cent NaOH solution at 120°C. for 5 hours; the extract was filtered off and the residue again extracted with fresh alkali solution. The combined extracts were neutralized with HCl, made slightly acid, and boiled to remove the extracted hemicelluloses and as much of the nitrogenous complexes as came down with the lignin. The lignin was now washed with water until free from acid and dried at 70°C. The air-dried lignin preparation had the following composition:

	per cent
Moisture	3.00
Ash	0.08
Nitrogen	0.25
Hemicellulose	0.85
Free lignin	94.45

When treated with 10 volumes of 80 per cent sulfuric acid for several hours in the cold, then diluted with 15 volumes of water and steamed for 5 hours, 90 per cent of this alkali lignin was found to be equivalent to acid lignin. In some of the experiments on the influence of lignin upon protein decomposition by microörganisms, acid lignin, prepared from straw or from wood, was also used.

As a source of protein, casein purified according to the method of Hammarsten, gliadin prepared from wheat flour by the well-known procedures, and egg-albumen were employed. The casein contained 9.3 per cent moisture and 15.87 per cent nitrogen on a dry basis, and the gliadin contained 5.5 per cent moisture and 15.35 per cent nitrogen on a dry basis.

Influence of lignin upon the decomposition of proteins by microörganisms

Four parts of lignin and one part of protein were thoroughly mixed in a dry state and aliquot portions (to give 1 per cent of protein) of the mixture added

TABLE 1

Influence of lignin upon the decomposition of proteins by pure and mixed cultures of microörganisms

Nitrogen liberated as ammonia in 11 days from 500 mgm. of protein material.

ORGANISM	NITROGEN LIBERATED AS AMMONIA FROM			
	Casein alone	Casein + lignin	Gliadin alone	Gliadin + lignin
	mgm.	*mgm.*	*mgm.*	*mgm.*
Control	0.28	1.26	0.56	1.12
Soil infusion	40.88	20.30	37.21	25.48
Bac. cereus	36.82	4.20	31.50	3.64
Actinomyces sp.	18.87	1.40	18.34	1.12
Trichoderma sp.	21.56	22.26	18.06	16.47

to 50-cc. portions of a liquid medium; the latter was sterilized, inoculated either with a soil infusion or with pure cultures of microörganisms, and incubated for 11 days, at the end of which time the ammonia-nitrogen was determined by distillation with MgO. The basic medium consisted of:

K_2HPO_4	1.0 gm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.
NaCl	0.1 gm.
$FeSO_4$	0.2 gm.
Distilled water	1,000 cc.
Reaction	pH 7.0

The results presented in table 1 show that a mere mechanical admixture of lignin and protein had, in many cases, a marked depressing effect upon ammonia formation. Even the mixed soil microbial population liberated only about a half as much nitrogen, from the decomposition of the proteins, in the presence of lignin as in its absence. In the case of the pure cultures of the

bacterium and actinomyces, the depressive effect of the lignin was especially marked, since the protein decomposition, as measured by ammonia liberation, was reduced to a minimum and frequently almost depressed entirely. However, in the case of the fungus, the presence of lignin exerted no injurious effect at all, or was very limited.

In order to determine whether it is the property of only the alkali lignin to depress the decomposition of the protein, or of lignin in general, acid lignin and alkali lignin were used in the following experiment. Varying amounts of two proteins and of the two lignins were added to 100-gm. portions of washed

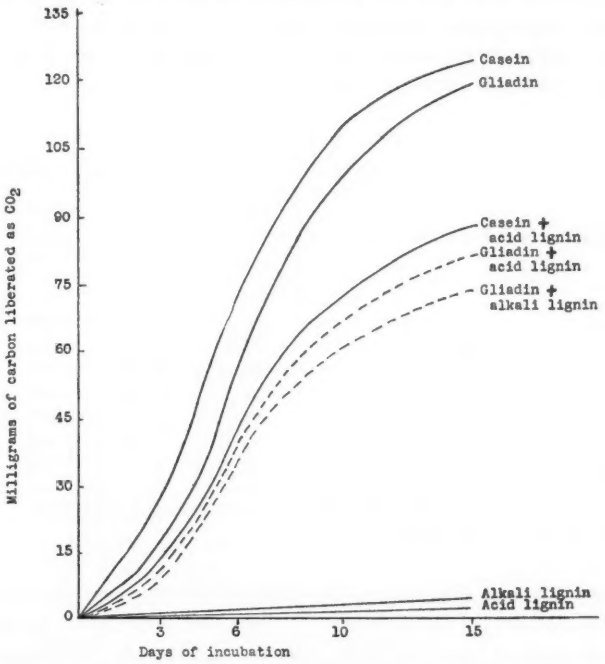

FIG. 1. INFLUENCE OF ACID-LIGNIN AND ALKALI-LIGNIN UPON THE DECOMPOSITION OF CASEIN AND GLIADIN BY MICROÖRGANISMS IN SAND MEDIUM, AS SHOWN BY THE LIBERATION OF CO_2

sand containing sufficient moisture to make conditions aerobic; various inorganic salts were also added. When protein and lignin were employed, the two were mixed mechanically by hand. The cultures were inoculated with soil infusion and incubated for 15 days. The flasks were connected with a respirator so as to measure the course of evolution of CO_2 during the decomposition of the protein, in the presence and absence of lignin. At the end of the incubation period, the ammonia produced was determined in an aliquot portion of the culture. To establish whether the protein has combined with the lignin

to form a "humus" complex (α-humus) (24), the residual material was extracted with 4 per cent NaOH solution; the extract was precipitated with HCl, filtered, washed, dried, and weighed; the nitrogen content of this "humus" was then determined. The results obtained are presented in table 2 and fig. 1. These results bring out very definitely the fact that both acid and alkali lignin have a marked depressive effect upon the decomposition of proteins in sand media, under favorable conditions and by a mixed microbial soil population. Further, the results point definitely to the formation of a complex between lignin and protein; this complex is equivalent to "humic acid" or to α-humus, in its solubility in alkalies and precipitation by acids, as well as in its nitrogen content. The amount of nitrogen found in this complex is nearly equivalent to the reduction in the amount of protein decomposed, in the presence of the lignin, as shown by the lower ammonia formation.

TABLE 2

*Influence of acid and alkali lignin upon the decomposition of proteins in sand medium**

SOURCE OF PROTEIN†	SOURCE OF LIGNIN	CONCENTRATION OF LIGNIN	CARBON DIOXIDE EVOLUTION, AS CARBON	NITROGEN LIBERATION, AS AMMONIA	α-HUMUS IN RESIDUAL MATERIAL	NITROGEN CONTENT OF α-HUMUS
		gm.	*mgm.*	*mgm.*	*gm.*	*mgm.*
Casein	0	...	121.7	56.70	0.048	1.82
Casein	Acid lignin	2.5	85.0	44.80	0.415	10.24
Gliadin	0	...	120.5	54.88	0.055	1.68
Gliadin	Alkali lignin	2.5	72.9	42.42	1.033	18.09
Gliadin	Acid lignin	2.5	81.3	44.80	0.395	10.54
0	Alkali lignin	1.0	3.1	0.56	0.400	1.12
0	Acid lignin	1.0	2.7	0.28	0.185	0.98

* Incubation 15 days; mixed soil infusion used for inoculation of all cultures.

† One-half gram of protein used.

In the following experiment, the casein and lignin were dissolved in 4 per cent NaOH solution (by warming), the solutions were mixed, so as to give 3 parts of lignin to 1 part of casein, and the mixture was acidified with hydrochloric acid. The precipitate formed was filtered off, washed with water until free from acid, and dried. The complex, in quantities equivalent to 500 mgm. of casein, was added to solution media similar to those described in the foregoing and to sand media, containing optimum moisture so as to give aerobic conditions and the necessary inorganic nutrients; the media were sterilized, inoculated, and incubated for 11 days. The results given in table 3 show that both in liquid and in sand media there was practically no decomposition of the casein, present in the form of the lignin-casein complex, as shown by ammonia liberation. This experiment was repeated, using other preparations and a 30-day period of incubation, with similar results.

To throw further light upon the effect of varying amounts of lignin and

upon the influence of sterilization of the medium upon the decomposition of the protein, the results obtained in the following experiment are reported. One-half gram portions of casein and varying amounts of lignin were added to 50-cc. quantities of solution medium containing the mineral salts; in some cases, the casein was used in the form of the lignin-casein complex, prepared

TABLE 3

Decomposition of lignin-casein complex by pure and mixed cultures of microörganisms

Nitrogen liberated as ammonia from 2 gm. of the complex containing about 500 mgm. of casein.

ORGANISM	NITROGEN LIBERATED AS AMMONIA FROM	
	Liquid medium	Sand medium
	mgm.	*mgm.*
Control	1.12	0.70
Soil infusion	2.52	1.96
Bac. cereus	1.12	0.84
Actinomyces sp	1.12	0.70
Trichoderma sp	1.12	0.84

TABLE 4

Influence of varying quantities of lignin upon the decomposition of casein by a mixture of soil microörganisms

Ammonia liberated from 500 mgm. of casein

AMOUNT OF LIGNIN USED	TREATMENT	STERILIZED	AMMONIA-NITROGEN FORMED
gm.			*mgm.*
None	Casein	+	42.0
None	Casein alone	−	42.4
1	Casein and lignin mixed mechanically	+	2.52
1	Casein and lignin mixed mechanically	−	31.72
2	Casein and lignin mixed mechanically	+	2.24
2	Casein and lignin mixed mechanically	−	23.75
2	Casein and lignin dissolved in alkali solutions, mixed in the cold and precipitated with acid	+	1.68
2	Casein and lignin dissolved in alkali solutions, mixed in the cold and precipitated with acid	−	1.68

according to the procedure outlined in the foregoing, four parts of lignin being used to one part of casein. Some of the cultures were sterilized and others were left unsterilized. All the cultures were inoculated with soil infusion. At the end of 11 days' incubation, the ammonia nitrogen was determined (table 4). The results show that the mere addition of lignin to the casein had a depressing effect upon the decomposition of the latter, as measured by

ammonia liberation, this effect increasing with an increase in the relative concentration of the lignin. When the medium was sterilized, the depressing effect was especially marked, since the heating resulted in the caking up of the lignin and led to the formation of a casein-lignin complex. When such a complex was produced by mixing alkali solutions of the two materials in the cold, and reprecipitating the complex by means of acid, the decomposition of the casein was stopped almost completely.

In order to obtain further information on the influence of increasing quantities of lignin upon the decomposition of casein, varying quantities of the two complexes were mixed in the form of dry powders and introduced into 50-cc. portions of the liquid medium. The media were left unsterilized, and were inoculated with a soil infusion. After 11 days' incubation at 28°C., the amounts of ammonia produced were determined (table 5). The results prove quite conclusively that increasing concentrations of lignin have a depressing effect upon the decomposition of the casein and upon the liberation of the

TABLE 5

Influence of increasing amounts of lignin upon the decomposition of casein by a mixed soil population

CASEIN USED	LIGNIN USED	AMMONIA-NITROGEN LIBERATED
gm.	*gm.*	*mgm.*
0.5	0	44.52
0.5	0.5	39.20
0.5	1.0	35.70
0.5	2.0	29.82
0.5	4.0	15.96
0.5	8.0	12.74

nitrogen in the form of ammonia by a mixed soil population, even when the medium is left unsterilized.

The following experiment deals also with the influence of varying concentrations of lignin upon the decomposition of casein by a mixed soil population in a non-sterilized liquid medium (50 cc.), containing inorganic salts and adjusted to pH 7.0. The two complexes were dissolved in normal solutions of sodium hydroxide and mixed in varying proportions. The mixed solutions were acidified to pH 4.5, which resulted in the formation of a heavy precipitate. This precipitate was filtered off, washed, dried at a low temperature, and used for the decomposition studies. In all cases sufficient casein solution was used to give 500 mgm. of the air-dry casein; however, when the alkali solution of the casein was adjusted to pH 4.5 by hydrochloric acid, not all the casein was precipitated out; the greater the relative amount of lignin used, the greater was the quantity of casein brought down in the complex, as shown by the total nitrogen content of the latter. The decomposition was allowed to proceed for 11 days and the ammonia was then determined.

The results (table 6) show definitely that the formation of a ligno-casein complex has a marked depressive effect upon the decomposition of the casein and the liberation of the nitrogen as ammonia. The greater the relative concentration of the lignin, when the protein concentration is kept constant, the less is the relative decomposition of the protein. The formation of a complex between the casein and the lignin is brought out by the fact that with an increasing relative concentration of lignin, the amount of casein removed from solution was increased. It remains to be determined whether we are dealing here with a physical or adsorption complex or with a chemical complex possessing new properties, the most important of which is the increasing resistance of the lignin to decomposition by microörganisms. Even when the lignin concentration was only one-fifteenth or one-tenth of the amount of casein in solu-

TABLE 6

Influence of varying concentrations of lignin and casein in the casein-lignin complex upon its decomposition by a mixed soil microbial population

COMPOSITION OF COMPLEX		YIELD OF PRECIPITATE	TOTAL NITROGEN IN COMPLEX*	AMMONIA-N LIBERATED	PER CENT OF TOTAL NITROGEN IN THE CASEIN LIBERATED AS AMMONIA
Casein used	Lignin used				
gm.	*gm.*	*gm.*	*mgm.*	*mgm.*	
0.500	0	0.200	21.56	8.40	39.0
0.500	0.010	0.232	23.80	8.12	34.1
0.500	0.050	0.247	23.88	7.70	32.2
0.500	0.200	0.517	32.48	6.58	20.3
0.500	0.500	0.849	41.25	2.80	6.8
0.500	1.250	1.540	43.50	0.14	0
0.500	2.500	2.750	47.60	0.14	0
0.500	5.000	5.224	56.45	0.14	0
0	0.500	0.501	2.80	0.14	0

* The nitrogen derived from the casein; the small amount of nitrogen present in the lignin was subtracted.

tion, there was already a sufficiently marked effect to reduce the casein decomposition, as measured by the liberation of nitrogen as ammonia. When the concentration of lignin was equivalent to that of the casein, the rate of decomposition of the latter was reduced to one-sixth of the relative rate of decomposition of the casein alone. When the concentration of lignin was two and a half times as great as that of casein, the decomposition of the latter was stopped completely.

There is no doubt that the protein can be absorbed from its solution by lignin, as shown in table 7. In this experiment, 50-cc. portions of casein solutions, equivalent to 1 gm. of air-dry casein, were adjusted to near neutrality and treated with varying quantities of moist lignin, which had been washed practically free from acid (pH 4.0) but not dried. The suspension of lignin and casein was shaken and allowed to stand in the cold for several hours,

filtered, washed, and the amount of nitrogen removed from the solution in the form of protein determined. The results (table 7) show that this is nearly proportional to the amount of lignin used.

It was essential to determine whether lignin has a depressive effect upon the decomposition of protein degradation products, such as peptones, polypeptides, and amino acids, in a manner similar to that exerted upon the decomposition of native proteins. This problem, apart from its theoretical significance, has also important practical applications, as in the decomposition of stable manure and green manures, in which a large part of the nitrogen is

TABLE 7

Absorption of protein from neutral solution by different amounts of alkali lignin in the cold

Fifty cubic centimeters of casein solution containing 1 gm. of air-dry casein was used in all cases.

AMOUNT OF LIGNIN USED	REACTION OF CASEIN SOLUTION OR OF FILTRATE	CASEIN REMOVED	
*gm.**	*pH*	*mgm. N*	*per cent of total N*
0	7.15	0	0
1	6.20	22.9	15.8
2	6.06	37.1	25.6
3	5.96	48.3	33.3

* On dry basis.

TABLE 8

Influence of lignin upon the decomposition of peptone and amino acids by microörganisms

NITROGEN SOURCE	LIGNIN	CARBON LIBERATED AS CO_2	NITROGEN LIBERATED AS AMMONIA
	gm.	*mgm.*	*mgm.*
1 gm. peptone	0	238.6	98.35
1 gm. peptone	4	243.6	94.85
1 gm. glutamic acid	0	241.9	71.05
1 gm. glutamic acid	4	243.0	69.65
1 gm. glycocoll	0	75.3	83.85
1 gm. glycocoll	4	156.7	123.55

present in the form of simple protein degradation products or protein building stones. Definite amounts of alkali lignin were mixed with peptone (Difco), glutamic acid, or glycocoll, in the proportion of four parts of lignin to one part of the nitrogenous complexes. The mixtures were placed in flasks containing 100-gm. portions of sand and the necessary inorganic salts, inoculated with a soil infusion, and connected with the respiration apparatus. The amount of CO_2 liberated in the process of decomposition was measured. At the end of the incubation period (21 days), the ammonia formed was determined (table 8).

The resultant effect of lignin upon the decomposition of peptone, as measured by evolution of CO_2 and accumulation of ammonia was neutral, i.e.

neither injurious nor beneficial. The same was true of its effect upon the decomposition of glutamic acid; however, in the case of glycocoll decomposition, the effect of lignin was beneficial. In this respect, the action of the lignin upon the decomposition of amino acids was similar to that exerted upon other microbiological processes, as reported later. These results thus prove beyond any doubt that the injurious effect of lignin upon protein decomposition holds true only for native proteins.

Synthesis of ligno-protein complexes and their decomposition

The results already presented seem to point to the formation between lignin and protein of complexes, which have properties distinctly different from both constituents. The purpose of the following experiments is to study the nature of these complexes, their formation under conditions similar to those which prevail in nature, their chemical and physico-chemical properties, their decom-

TABLE 9

Influence of reaction and bases upon the precipitation of the ligno-protein complex

REACTION	BASE USED	WEIGHT OF PRECIPITATE*	TOTAL NITROGEN IN PRECIPITATE†
pH		*gm.*	*mgm.*
5.5	H^+	2.620	56.10
5.0	H^+	2.857	65.58
4.5	H^+	2.895	72.50
4.0	H^+	2.940	73.67
6.0	Mg^{++}	3.615	60.70
6.0	Ca^{++}	3.520	62.72
8.0	Ca^{++}	3.580	62.15

* Five hundred milligrams casein and 2.5 gm. of lignin used, air-dry material.

† The ash content was 1.4 per cent for the hydrogen preparation and 15.0 per cent for the calcium preparation; an iron complex was also prepared containing 4.6 per cent iron.

position, and their influence upon the growth and activities of microörganisms. The relation of these complexes to the various bases which commonly occur in soil presented a most interesting problem. In view of the fact that the reaction of the soil is definitely dependent upon the nature and concentration of the soil bases, the influence of reaction upon the precipitation of the ligno-protein complex, as well as the influence of ions of heavy metals, especially of Ca and Mg, upon the formation of this complex was determined. Alkali solutions of casein and lignin, in the ratio of 5 parts of lignin to 1 part of casein were mixed. Definite quantities of the two solutions were placed in a series of flasks and enough HCl added to adjust the reaction to pH 5.5, 5.0, 4.5, and 4.0, respectively. In the absence of salts, practically no precipitate was formed at pH 5.5 or above; at pH 4.0, practically the whole complex was precipitated out. When aqueous solutions of $CaCl_2$ and $MgCl_2$ were added, the whole ligno-casein complex was precipitated out at much lower hydrogen-

ion concentrations (pH 5.5–8.0). The precipitates were washed free from salts with water, dried, weighed, and analyzed for total nitrogen (table 9).

These preparations were added to liquid media containing mineral salts, inoculated with a soil suspension, and incubated. None of the cultures showed any signs of decomposition in 11 days, as measured by ammonia formation; very little microbial growth could be found by macroscopic and microscopic observation. This definitely pointed to the fact that, as a result of the interaction of lignin with protein, complexes had been formed consisting of a certain concentration of base, of lignin, and of protein; the protein was thus inactivated almost quantitatively against decomposition by microörganisms.

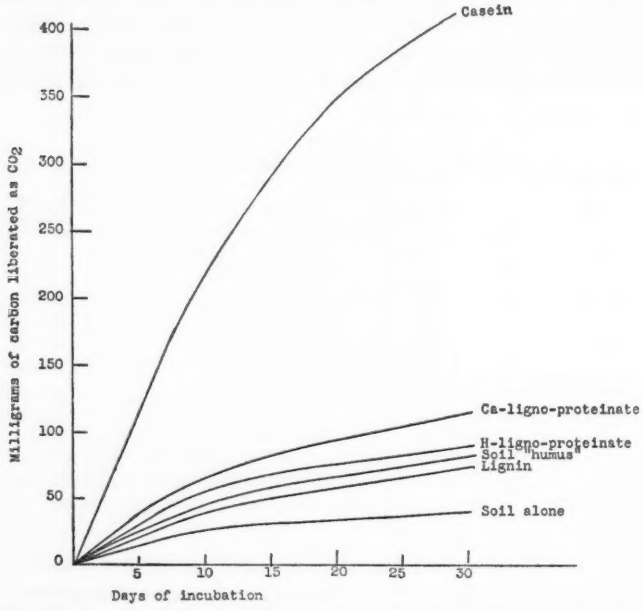

FIG. 2. COURSE OF DECOMPOSITION OF CASEIN, LIGNIN, SOIL HUMUS, AND LIGNO-PROTEIN COMPLEXES IN SOIL, AS MEASURED BY THE EVOLUTION OF CO_2

The experiments on the decomposition of the ligno-protein complexes have been carried out so far in solution and in sand media, for comparatively short periods of time. It is essential of course to determine the behavior of these complexes in the soil. Two-hundred gram quantities of a Sassafras loam soil were placed in a series of Erlenmeyer flasks. Some of the soils were left untreated and only adjusted to optimum moisture content; some received 6-gm. portions of alkali lignin, some received 6-gm. portions of the H-ligno-protein complex, and some received 6-gm. portions of the Ca-ligno-protein complex. For the purpose of comparison, 4.5-gm. portions of α-humus, ob-

tained from soil by extraction with alkali and precipitation with acid, were added to a series of flasks; some of the flasks received 0.95-gm. portions of pure casein. The nitrogen content of the casein used was 136.8 mgm., of the H-ligno-proteinate 136.08 mgm., of the Ca-ligno-proteinate 134.4 mgm., of the α-humus 134.8 mgm., and of the lignin 20.16 mgm. Sufficient water was added to bring the soils to optimum moisture. The flasks were connected with the respiration apparatus and the amount of CO_2 evolved was determined (fig. 2). At the end of 30 days' incubation, the ammonia and nitrate contents in the soils were measured. A portion of the soil was extracted with 4 per cent sodium hydroxide solution, the extract filtered and the filtrate acidified with hydrochloric acid; the precipitate thus formed was removed, washed with water, and dried.

The results (table 10) show that free lignin was decomposed in the soil only to a limited extent; this decomposition was accompanied by a reduction in the

TABLE 10

Decomposition of lignin and ligno-protein complexes in soil, as measured by CO_2 evolution, nitrogen liberation, and humus formation

SOIL TREATMENT	CO_2 LIBERATION		NITROGEN LIBERATION			α-HUMUS	
	15 days	30 days	NH_3	NO_3	Total N	Yield	Excess over control
	mgm. C	*mgm. C*	*mgm. N*	*mgm. N*	*mgm.*	*gm.*	*gm.*
Soil alone	29.8	41.0	0	8.96	8.96	1.304	.....
Soil + lignin	49.8	72.7	0	5.60	5.60	7.077	5.773
Soil + α-humus	53.5	78.2	6.16	4.48	10.64	5.496	4.192
Soil + H-ligno-proteinate	59.4	86.2	1.12	9.52	10.64	7.011	5.707
Soil + Ca-ligno-proteinate	76.7	112.1	0.98	10.08	11.06	6.927	5.623
Soil + casein	293.5	399.3	80.36	29.40	109.76	1.435	0.131

amount of nitrogen liberated as nitrate in the soil, no doubt as a result of its consumption by the microörganisms decomposing the lignin. The α-humus isolated from ordinary field soil, or the typical "humic acid," decomposed at about the same rate as the lignin, or also very slowly. However, in view of the fact that the C:N ratio of this complex has become stabilized, with a nitrogen content of a little over 3 per cent, typical of such preparations, the decomposition of the complex should logically be accompanied by a liberation of nitrogen in an available form. An increase in decomposition above the control soil, as measured by the liberation of additional 31.7 mgm. of carbon as CO_2 in 30 days, was accompanied by an increase of 1.68 mgm. in the liberation of nitrogen as ammonia.

The decomposition of the ligno-protein complexes is most interesting. The H-ligno-proteinate decomposed at about the same rate as the α-humus of the soil, as shown both by the CO_2 evolution and the nitrogen liberation. The Ca-ligno-proteinate decomposed somewhat more rapidly. However, the rate

of decomposition of this complex presented a marked contrast to the decomposition of the 0.95 gm. of casein, containing exactly the same amount of protein. One cannot doubt here the fact that a stable complex between the lignin and the protein has been produced, and that this complex behaves, as far as its decomposition in the soil is concerned, as typical soil "humus." This is further substantiated by the extraction of the "humus" from the soil by means of an alkali solution and its precipitation by means of hydrochloric acid. The α-humus used in this experiment was practically all recovered in the "soil humus" fraction; the same was exactly true of the synthesized ligno-protein complexes.

Chemical nature of the ligno-protein complexes

For the purpose of studying further the nature of the complex formed between casein, lignin, and various bases, especially for the purpose of establishing whether we are dealing here with a stable chemical complex or with a

TABLE 11

Solubility of alkali lignin, ligno-proteins, and "soil humus" in cold and hot alcohol and in acetone

PREPARATION*	MATERIAL SOLUBLE IN COLD ALCOHOL	MATERIAL SOLUBLE IN HOT ALCOHOL	TOTAL ALCOHOL-SOLUBLE	TOTAL NITROGEN IN ORIGINAL PREPARATION*	NITROGEN CONTENT OF ALCOHOL-SOLUBLE PORTION	SOLUBLE IN HOT ACETONE	NITROGEN CONTENT OF ACETONE-SOLUBLE PORTION
	per cent	*per cent*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*
Lignin	48.1	10.3	58.4	8.82	7.21	66.1	5.76
H-ligno-proteinate	38.6	10.7	49.3	63.00	31.01	49.1	11.04
Ca-ligno-proteinate	1.2	1.2	2.4	59.64	.....	0.3	....
α-humus from peat	22.8	14.0	36.8	88.80	20.95	20.1	4.68

* Three grams of air-dry material.

physical or absorption complex, several preparations were subjected to a detailed chemical analysis. This consisted in determining the composition of these preparations, their behavior to different solvents, their oxidation capacity, their reactivity with acetyl bromide, etc. Three-gram portions of several of the preparations were at first extracted with cold 95 per cent alcohol for 24 hours, followed by extraction under a reflux condenser with hot alcohol for 2 hours. The extracts were removed by filtration and evaporated in weighed dishes; a portion of the extract was then analyzed for total nitrogen. Similar extraction was made with hot acetone for 2 hours.

The results presented in table 11 point definitely to the formation of a stable complex between the lignin and the protein, similar in its nature to "soil humus." The lignin preparation was readily soluble in alcohol and in acetone; however, the ligno-protein complexes were less soluble, especially the calcium complex, which was practically insoluble in both acetone and alcohol. This may explain the commonly observed phenomenon that soil humus gives very

little alcohol-soluble material, while it is still present in the soil, but once it has been extracted by an alkali solution and precipitated by an acid, a large part of the humus is found to be alcohol soluble, as shown in the case of the α-humus preparation. More important than this is the fact that, in the case of the H-complex, a large part of which is soluble in alcohol, the percentage of nitrogen in the soluble portion is the same as in the initial complex. This points to the chemical nature of the complex. Were the ligno-protein complex a mere physical mixture, one would expect that the lignin would become partly soluble in the alcohol, while the protein would remain insoluble; the fact that the alcohol extracted a proportional amount of the protein and of the lignin points to a certain chemical combination between the two.

TABLE 12

Oxidation of alkali lignin, ligno-proteins, and "soil humus" by 6 per cent H_2O_2

PREPARATION	MATERIAL OXIDIZED
	per cent
Lignin	7.2
H-ligno-proteinate	17.6
Ca-ligno-proteinate	38.3
α-humus from peat	75.8

TABLE 13

Oxidation of synthesized and natural "humus" complexes by 6 per cent H_2O_2

PREPARATION	RATIO OF LIGNIN TO CASEIN	AMOUNT OXIDIZED
		per cent
Lignin	1:0	4.9
H-ligno-proteinate	8:1	11.8
H-ligno-proteinate	4:1	13.1
H-ligno-proteinate	2:1	17.4
α-humus from mineral soil	...	28.2
α-humus from lowmoor peat	...	95.6
Electrodialyzed humus	...	46.1

In order to determine to what extent the ligno-protein complexes are readily oxidized with weak oxidizing agents, the various preparations were treated with hot 6 per cent H_2O_2 (table 12). It should be recalled that this reagent has been recommended for determining the "humified" part of the soil organic matter. The results show that the ligno-protein complexes are "humified," since they are attacked readily by this reagent. The chemical nature of the preparation has a marked influence upon the extent of its oxidation; in this respect different "humus" preparations from soil behave similarly, as shown in table 13.

The older literature is full of references to determinations of "humus" by extracting the soil with ammoniacal solution, evaporating the extract, and

weighing the residue. This procedure, originally suggested in 1872 by Grandeau, giving "matière noire" or black matter, was later variously modified; it probably served more than any other procedure in confusing rather than in advancing our knowledge of the nature and origin of soil humus. Without the least consideration being given to the nature of the complexes extracted by this reagent, thousands of determinations of "humus" were made all over the world. In order to illustrate the bearing that this method may have upon the synthesized humus complexes, the various preparations were extracted for 24 hours with 4 per cent NH_4OH solution. The extracts were filtered and evaporated; the residues were dried and weighed (table 14). The results show that practically all the lignin, the H-ligno-proteinate and the α-humus from peat were soluble in cold ammonium hydroxide solution, whereas only one-fifth of the Ca-complex was dissolved by this reagent. This is the reason why in all the determinations of the "humus" by the Grandeau method, it was always recommended to treat the soil first with hydrochloric acid, which results in

TABLE 14

Solubility of lignin, ligno-proteins, and "soil humus" in cold ammonium hydroxide solution

PREPARATION	SOLUBLE IN NH_4OH
	per cent
Lignin	97.5
H-ligno-proteinate	97.4
Ca-ligno-proteinate	19.2
α-humus from peat	97.4

the dissolution of the calcium or the substitution in the complex of hydrogen for the calcium.

It is well known that although proteins are readily hydrolyzed by hot dilute mineral acids, especially after prolonged boiling, the organic nitrogenous compounds of the soil are only partly hydrolyzed. This gave rise to the assumption that some of the nitrogen in the soil humus is not of a protein nature, but is present in the form of ring compounds, or in the form of "humin-nitrogen," whatever that may be. In order to compare further the synthesized complexes with soil humus, 3-gm. portions of the various preparations were treated with 100 cc. 5 per cent hydrochloric acid solution, at 100°C. for 2 hours; the nitrogen thus rendered soluble was then determined (table 15). The results reported show that whereas over 80 per cent of the nitrogen in the casein was brought into solution by dilute acid hydrolysis, only 20 to 33 per cent of the nitrogen was rendered soluble in the case of the ligno-proteinate and about 30 per cent in the case of the "soil humus." The artificial humus preparations behaved again in exactly the same manner as the natural soil humus.

These results seemed so important that a number of other preparations were treated with dilute hydrochloric acid (2 per cent), for 2 hours at 100°C. and

with hot 95 per cent alcohol (2 hours at 100°C.); these data were compared with the decomposition of the various preparations in soil. For this study, 3-gm. portions of the various preparations were added to 100-gm. portions of field soil; the cultures were incubated for 32 days and the CO_2 liberation was measured. At the end of the incubation period, the amount of nitrate produced was determined. The results (table 16) obtained fully confirm the previous observations.

TABLE 15

Influence of acid hydrolysis upon the ligno-proteins and "soil humus"

PREPARATION	UNHYDROLYZED RESIDUE	PER CENT OF PROTEIN NITROGEN HYDROLYZED
	per cent	
Casein	6.8	80.6
Lignin	94.3	3.2
H-ligno-proteinate	89.3	32.9
Ca-ligno-proteinate	80.3	20.2
α-humus from peat	83.0	29.9

TABLE 16

Influence of acid hydrolysis, treatment with hot alcohol, and microbial decomposition upon several ligno-protein preparations

NATURE OF PREPARATION	NITROGEN CONTENT	HYDROLYSIS WITH HCl		SOLUBILITY IN 95 PER CENT ALCOHOL		DECOMPOSITION BY MICROÖRGANISMS IN SOIL	
		Per cent of total preparation hydrolyzed	Per cent of total nitrogen hydrolyzed	Per cent of total preparation brought into solution	Per cent of total nitrogen brought into solution	CO_2 liberated	Nitrate formed
	per cent					*mgm. C*	*mgm. N*
Lignin alone	0.12	2.0	45	13.6	....	44.1	1.3*
H-ligno-caseinate	2.68	11.2	32	41.8	33.4	46.1	3.2
H-ligno-albuminate	3.01	12.8	35	47.0	14.0	40.6	2.5
Fe-ligno-caseinate	2.96	13.7	32	39.2	18.1	48.9	2.2
Fe-ligno-albuminate	2.91	6.7	16	....	....	56.6	2.5
α-humus from peat	2.94	23.7	23	....	....	....	...

* Control soil liberated 37.5 mgm. carbon as CO_2 and 2.3 mgm. NO_3—N.

Finally one crucial experiment may be reported here, namely, the behavior of the artificial humus preparations and of natural humus to the acetyl bromide reagent. Karrer and Boding-Wieger (12) found that acetyl-bromide dissolves all plant constituents, but does not act upon "humic bodies"; they suggested, therefore, the separation of humus from undecomposed plant materials by means of this reagent and its determination quantitatively. Groszkopf (9) and Springer (21) used this reagent extensively for the determination of the

"humus" content in soil and in peat. The fact that certain humus complexes, namely, the so-called "hymetomelanic acid" or the alcohol-soluble "humic acid," are oxidized by acetyl-bromide led Groszkopf to consider the latter as lignin-like in nature.

One-gram portions of the various preparations were treated with 50-cc. portions of acetyl-bromide at 50°C. for 8 hours. At the end of that period of time, the extracted material was filtered through Gooch crucibles, the residue was dried, washed with ether, dried again, and weighed (table 17). The results show that whereas lignin is nearly completely dissolved by acetyl-bromide, the α-humus from peat is attacked only to a limited extent. The ligno-proteinates stand midway, the hydrogen complex being acted upon to the extent of nearly 90 per cent and the calcium complex to about 53 per cent. These results as well tend to show that as a result of interaction of the protein, lignin, and base, a new complex is formed which is more resistant to the action of acetyl-bromide than the original constituents; this complex tends to approach in this respect as well the humus of the soil.

TABLE 17

The action of acetyl-bromide upon lignin, ligno-proteins, and "soil humus"

PREPARATION	MATERIAL ATTACKED BY ACETYL-BROMIDE
	per cent
Lignin	99.0
H-ligno-proteinate	89.8
Ca-ligno-proteinate	53.2
α-humus from peat	21.4

DISCUSSION

The results of the experiments presented, as well as others which will be published in one of the following contributions to this subject, amply illustrate the fact that by the interaction of lignin and protein a complex is formed which possesses all the characteristic properties of the major fraction of the humus of the soil, namely, that part which is soluble in alkalies and is precipitated by acids, and which has commonly been referred to as "humic acid." The comparative characteristic properties of this complex with that of α-humus or "humic acid" of the soil can be briefly summarized as follows:

PROPERTY OR REACTION	α-HUMUS OF SOIL OR PEAT	HUMUS-NUCLEUS OR LIGNO-PROTEINATE
Nitrogen content	2–3 per cent	2–3 per cent
Combination with bases	Combines readily	Combines readily
Solubility in dilute alkali solutions	Soluble	Soluble
Solubility in dilute mineral acids	Insoluble	Insoluble
Oxidation by H_2O_2	Readily oxidized	Readily oxidized
Oxidation by acetyl bromide	Only slightly oxidized	Not fully oxidized
Decomposition by microörganisms	Attacked with difficulty	Attacked with difficulty
Influence on microbial processes	Favorable	Favorable

Figure 3 gives a schematic presentation of the mechanism of humus formation, as a result of the decomposition of plant residues, on the basis of these and other investigations carried out in this laboratory. The assumption is thereby made that soil humus, or the total soil organic matter, consists of a humus-nucleus, resistant to decomposition, and accompanying substances, which consist of certain plant constituents and synthesized complexes undergoing decomposition. It is, of course, understood that the formation by condensation of a ligno-protein compound, or of a humus nucleus, can account for only a part of the humus in average soils. A number of chemical reactions in connection with the soil organic matter cannot be explained by the presence of these compounds alone. It is sufficient to mention the uronic acid com-

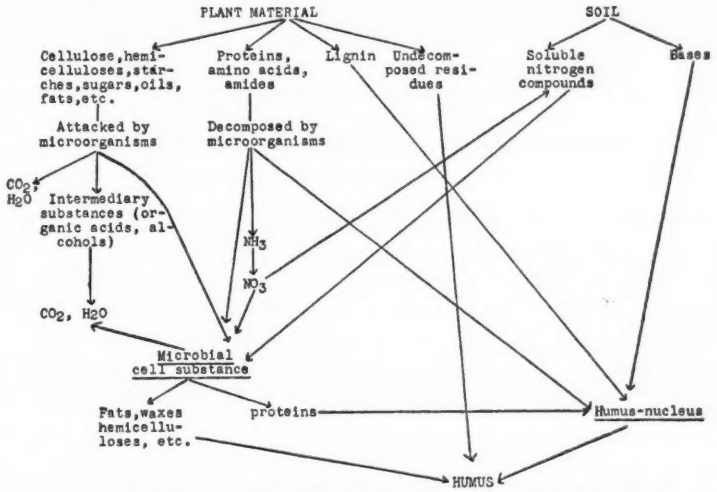

FIG. 3. SCHEMATIC REPRESENTATION OF THE MECHANISM OF HUMUS FORMATION IN THE DECOMPOSITION OF PLANT RESIDUES IN THE SOIL

plexes in soil and the presence of various aldehydes, acids, and alcohols (19, 20). Further, in view of the fact that proteins, varying considerably in chemical composition, enter the soil in the plant and animal residues or are formed there through the activities of the microörganisms, while lignins may also vary considerably in chemical composition, the nature of the ligno-protein complexes formed under different conditions will vary. The nature of the base seems to be also of considerable importance in modifying the chemical nature and reactivity of the complexes. Although the results reported here deal largely with the reactions of the hydrogen and calcium complexes, the information which is now being accumulated and which will be reported later on the behavior of the iron and aluminum compounds, shows that these approach, in their characteristics, the soil humus even more than the other two complexes.

As to the chemistry of the reaction of condensation involved between the protein and the lignin, several possibilities suggest themselves. These are based upon the fact that lignin is known to possess carboxyl groups, hydroxyl groups, carbonyl groups, methoxyl groups, in addition to the cyclic rings, or the ring skeleton (7). If one assumes that the COOH group of the lignin interacts with the NH_2 group of the protein, a salt formation will take place, even in an alkaline solution; a reaction of this type takes place in the process of tanning. The possibility of interaction between a phenolic OH group and the NH_2 group would meet with the objection that, aside from the improbability of such a reaction taking place, the complex itself could be readily hydrolyzed. The assumption of a reaction between the NH_2 group of the protein molecule with a carbonyl group, of a ketonic or an aldehydic nature, to form a compound of the nature of a Schiff's base, offers a fair degree of probability.

$$\underset{\text{Lignin}}{C_{52}H_{46}O_{10}(OCH_3) \cdot (COOH) \cdot (OH)_4 \cdot CO} + \underset{\text{Protein}}{H_2N \cdot R \cdot COOH} \rightarrow$$

$$\underset{\text{Humus-nucleus}}{C_{52}H_{46}O_{10}(OCH_3) \cdot (COOH) \cdot (OH)_4 \cdot C{=}N \cdot R \cdot COOH} + H_2O$$

This complex would be highly stable and would possess a high base exchange capacity, a phenomenon characteristic of the humus-nucleus. However, since the combining capacity of proteins with bases and acids cannot be fully accounted for by the free carboxyl or amino groups of the protein molecule and since the CO·HN groups in the interior of the molecule are capable of combination, giving compounds not readily subject to hydrolytic dissociation, various other combinations between proteins and lignins suggest themselves.

SUMMARY

1. Studies are reported concerning the influence of lignin upon the decomposition of proteins by pure and mixed cultures of microörganisms.

2. Lignin was found to exercise a depressive effect upon the decomposition of proteins; this depression is not due to any toxic action of the lignin, but is a result of the interaction of the lignin with the protein, which makes the latter more resistant to attack by microörganisms.

3. A resistant complex consisting of lignin and protein, two of the important constituents of plant, animal, and microbial residues, has been synthesized in the laboratory. This complex is similar in physical appearance and possesses the various chemical, physico-chemical and biological properties characteristic of the major portion of the soil organic matter, which is usually referred to as "humus" or "humic acid."

4. The ligno-protein complexes will combine with various bases, such as calcium, magnesium, iron, and aluminum, in a manner similar to the combination between soil humus and these bases.

5. In view of the fact that the major portion of the soil humus is made up of

lignin complexes, or their derivatives, and of protein complexes, it is suggested that the soil humus consists largely of these two compounds combined to form new chemical complexes.

6. The synthesized ligno-protein complex will be referred to as the "humus-nucleus," in contradistinction to the term "humus," which is reserved for the soil organic matter as a whole; the term "synthetic humus" cannot be used, since it has been commonly employed to designate the dark colored substances obtained on treatment of sugar with mineral acids, or by the oxidation of phenols.

7. The synthesis of the humus-nucleus enables one to carry out studies of the physical, chemical, and biological behavior of soil humus and its relation to the inorganic soil constituents, without having to use the complex humus of the soil itself, since this humus varies considerably in chemical composition, depending on the soil from which it is obtained and upon the method of isolation.

8. The formation of a humus-nucleus complex establishes definitely the relation between the organic nitrogenous substances and the non-nitrogenous substances of the soil humus; this is responsible for the resistance of the soil nitrogen to rapid decomposition by microörganisms. The formation of such a complex also suggests evidence to explain the more or less constant carbon-nitrogen ratio which exists in mineral soils.

9. The synthesis of the humus-nucleus points to an explanation for the relation between the organic complexes in the soil and the inorganic soil constituents, especially the soil bases. At a pH less than 4.8, the ligno-protein complex is largely saturated with hydrogen, iron, and aluminum. The higher the pH value, the greater will be the replacement of the H-ion by Ca and Mg ions and finally, at a pH of 9.0 or above, by Na-ions. At a reaction of pH less than 4.8, most of the complex exists as a hydrogen-ligno-proteinate and also as Fe- and Al-ligno-proteinates; at a pH of 4.8 to 9.0, it exists largely as Ca- and Mg-ligno-proteinates, and probably also as Fe- and Al-ligno-proteinates; and at pH above 9.0, especially at 9.6–10.0, it exists largely as a sodium-ligno-proteinate.

10. The exact chemical nature of the complex still remains to be studied; its nature varies with the nature of the lignin and proteins used, relative concentration of these two substances, reaction at which precipitation takes place, relative concentration and nature of bases, degree of drying, and a number of other factors.

REFERENCES

(1) Balks, R. 1925 Untersuchungen über die Bildung und Zersetzung des Humus im Boden. *Landw. Vers. Sta.* 103: 221–258.

(2) Berzelius, J. J. 1839 Lehrbuch der Chemie, ed. 3, Tr. by Wöhler, 8: 11–16, 384–431.

(3) Dehérain, P. P. 1888 Recherches sur les fermentations du fumier de ferme. *Ann. Agron.* 14: 97–133. (Traité de chimie agricole, Paris, 1902).

(4) Demolon, A., and Brigando, J. 1932 Sur la fixation des protéides par le sol. *Compt. Rend. Acad. Sci.* 194: 311–313.

(5) FISCHER, F., AND SCHRADER, H. 1922 Über die Entstehung und die chemische Struktur der Kohle. *Brennstoffchem.* 2: 37–45.

(6) FREUDENBERG, K., SOHN, F., DÜRR, W., AND NIEMANN, C. 1931 Ueber Lignin, Coniferylalkohol, und Saligenin. *Cellulosechemie.* 12: 263–276.

(7) FUCHS, W. 1926 Die Chemie des Lignins. J. Springer, Berlin.

(8) FUCHS, W. 1931 Untersuchungen über Lignin, Huminsäuren und Humine. *Ztschr. Angew. Chem.* 44: 111–134.

(9) GROSZKOPF, W. 1931 Fortschritte in der Humusforschung. *Südd. Forst.-u. Iagdztg,* p. 33–48.

(10) HOBSON, R. P. 1925 A study of the nitrogen compounds of mineral soils. Thesis. University London.

(11) JENSEN, H. L. 1931 The microbiology of farmyard manure decomposition in soil: I. Changes in the microflora, and their relation to nitrification. *Jour. Agr. Sci.* 21: 38–80.

(12) KARRER, P., AND BODDING-WIGER, B. 1921–1925 Kenntnis des Lignins. *Helvetica Chim. Acta.* 4: 700; 6: 817–821; Diss. Zürich, 1925.

(13) LATHROP, E. C. 1917 The organic nitrogen compounds of soils and fertilizers. *Jour. Franklin Inst.* 183: 169–206, 303–321, 465–498.

(14) LUNDIN, H., AND SCHRÖDERHEIM, J. 1931. Über die Fällung von Eiweiszstoffen und ihrer Spaltungsprodukte mit Tannin. *Biochem. Ztschr.* 238: 1–23.

(15) MCLEAN, W. 1931 The nature of soil organic matter as shown by the attack of hydrogen peroxide. *Jour. Agr. Sci.* 21: 595–611.

(16) MOELLER, W. 1916 Humussäure und Gerbsäure. Der Gerber. 1916. Nos. 1000, 1003, 1008, 1009; Collegium, 330–333, 356–359, 385–393, 452–462, 1916. [*Chem. zentbl.* II: 856 (1916); I: 30, 440 (1917)].

(17) ODEN, S. 1919 Die Huminsäuren. *Kolloid chem. Beihefte* 11: 75–260.

(18) RITTER, G. J. 1925 Distribution of lignin in wood. *Jour. Indus. and Engin. Chem.* 17: 1194.

(19) SCHREINER, O., AND SHOREY, E. C. 1909 The isolation of harmful organic substances from soils. U. S. Dept. Agr. Bur. Soils Bull. 53.

(20) SHOREY, E. C. 1928 Non-humus constituents of the humus extract. *Proc. First Internatl. Cong. Soil Sci.* (Washington) 3: 264–268.

(21) SPRINGER, U. 1931 Neuere Methoden zur Untersuchung der organischen Substanz im Boden und ihre Anwendung auf Bodentypen und Humusformen. *Ztschr. Pflanzenernähr., Düngung., u. Bodenk.* 22A: 135–152.

(22) DUTOIT, M. M. S., AND PAGE, H. J. 1930 Studies on the carbon and nitrogen cycles in the soil: III. The formation of natural humic matter. *Jour. Agr. Sci.* 20: 478–488.

(23) WAKSMAN, S. A. 1926 The origin and nature of soil organic matter or soil "humus:" I. Introductory and historical. *Soil Sci.* 22: 123–162.

(24) WAKSMAN, S. A. 1926 The origin and nature of soil organic matter or soil "humus:" II. Method of determining humus in the soil. *Soil Sci.* 22: 221–232.

(25) WAKSMAN, S. A. 1929 Chemical nature of soil organic matter, methods of analysis, and the rôle of microörganisms in its formation and decomposition. *Trans. Second Comn. Internatl. Soc. Soil Sci.* (Budapest) Part A: 172–197.

(26) WAKSMAN, S. A. 1930 Chemical composition of peat and the rôle of microörganisms in its formation. *Amer. Jour. Sci.* 19: 32–54.

(27) WAKSMAN, S. A., AND MCGRATH, J. M. 1931 Preliminary study of chemical processes involved in the decomposition of manure by *Agaricus campestris. Amer. Jour. Bot.* 18: 573–581.

(28) WAKSMAN, S. A., AND IYER, K. R. N. 1932 Synthesis of a humus-nucleus, an important constituent of humus in soils, peats and composts. *Jour. Wash. Acad. Sci.* 22: 41–50.

(29) WAKSMAN, S. A., AND STEVENS, K. R. 1930 A critical study of the methods and abundance of soil organic matter. *Soil Sci.* 30: 97–116.

(30) WEISS, FR. 1903 A study of the proteolytic enzymes of malt. *Compt. Rend. Lab. Carlsberg* 5: 133–285.

(31) WILLSTÄTTER, R. (Ref. by Rassow and Gabriel, *Cellulosechemie* 12: 227–235).

(32) WISLICENUS, R. 1925 Colloid chemistry of the synthesis and degradation of the cellulose framework of the plant, "lignin" and wood fiber. *Cellulosechemie* 6: 45–58. (*Chem. Abs.* 19: 2363).

(33) WOLLNY, E. 1897 Die Zersetzung der organischen Stoffe und die Humusbildungen mit Rücksicht auf die Bodencultur. C. Winter, Heidelberg.

CONTRIBUTION TO OUR KNOWLEDGE OF THE CHEMICAL NATURE AND ORIGIN OF HUMUS: II. THE INFLUENCE OF "SYNTHESIZED" HUMUS COMPOUNDS AND OF "NATURAL HUMUS" UPON SOIL MICROBIOLOGICAL PROCESSES[1]

SELMAN A. WAKSMAN AND K. R. N. IYER

New Jersey Agricultural Experiment Station

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The rôle of humus in soil processes has frequently been ascribed to its beneficial action upon the growth and activities of soil microörganisms. Among these, the influence of humus upon the growth of Azotobacter and other nitrogen-fixing bacteria has received particular attention (3). It was believed that this favorable effect of humus is due to its colloidal properties, but recent information tends to prove that this is due, both as regards plant growth (4) and the development of Azotobacter (1, 5), to the iron content of the humus.

Among the other phenomena concerned with the influence of humus upon microbial development, the extent to which humus can be used by microörganisms has also received considerable attention. Although the ability of bacteria and fungi to attack "humic acids" as sources of carbon has been denied, the utilization of these acids as sources of nitrogen was considered possible (6).

In order to study the influence that synthesized humus complexes may have upon microbiological activities, whether toxic, favorable, or neutral, as compared with that of natural soil humus, or organic compounds found in soil and peat, several microbiological processes, characteristic of the soil, were investigated.

The first experiment deals with the influence of humus upon the decomposition of glucose by microörganisms. The following medium was prepared:

	gm.		gm.
Glucose	20	NaCl	0.1
$NaNO_3$	2	$FeSO_4$	0.02
K_2HPO_4	1	Distilled water	1.000
$MgSO_4 \cdot 7H_2O$	0.5		

This medium was distributed into flasks, some of which received the lignoprotein complexes and others did not. Another medium of similar composition, but free from nitrate, was also prepared, so as to determine to what extent the

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nitrogen in the ligno-protein complexes can be used by the organisms decomposing the glucose. The media were sterilized, inoculated with soil infusion, and incubated for 6 days. At the end of that period, the remaining glucose in the cultures was determined. The results presented in table 1 show that

TABLE 1

Influence of lignin and ligno-proteinates upon the decomposition of glucose by microörganisms

TREATMENT OF CULTURE	NITROGEN ADDED	GLUCOSE LEFT	GLUCOSE DECOMPOSED
		mgm.	*mgm.*
Glucose alone, uninoculated*	−	974	0
Glucose alone, uninoculated	+	974	0
Glucose alone, inoculated	−	906	68
Glucose alone, inoculated	+	615	359
Glucose + lignin†	−	900	74
Glucose + lignin	+	773	201
Glucose + H-ligno-proteinate‡	−	917	57
Glucose + H-ligno-proteinate	+	643	331
Glucose + Ca-ligno-proteinate	−	720	254
Glucose + Ca-ligno-proteinate	+	607	367

* One gram of glucose in addition to the mineral nutrients.

† Three grams of lignin used per flask.

‡ One gram of the ligno-proteinates used.

TABLE 2

Influence of lignin, ligno-proteinates, and "soil humus" upon the decomposition of cellulose by microörganisms

SUBSTANCES USED	NITROGEN USED AS NITRATE	TOTAL CO_2 LIBERATED	CELLULOSE DECOMPOSED	NITRATE CONSUMED	RATIO OF CELLULOSE DECOMPOSED TO NITROGEN CONSUMED
		mgm. C.	*mgm.*	*mgm. N*	
Control medium*	0	9.0	47.7	.....	....
Ca-ligno-proteinate, 1 gm.	0	25.9	.....	.....	....
H-ligno-proteinate, 1 gm.	0	18.5	.....	.....	....
α-humus, 1 gm.	0	17.7	.....	.....	....
Control medium	+	132.7	438.5	13.78	31.8
Lignin, 2 gm.	+	185.0	522.0	16.12	32.4
Ca-ligno-proteinate, 1 gm.	+	185.3	552.3	16.56	33.4
H-ligno-proteinate, 1 gm.	+	159.0	480.2	14.76	32.5
α-humus, 1 gm.	+	159.4	468.7	14.48	32.3

* One-gram portions of cellulose used in each culture.

lignin alone had a somewhat depressing effect, in the presence of available nitrogen in the culture, upon the decomposition of the glucose. The H-ligno protein complex had only a very slight depressing effect, both in the presence and absence of added nitrogen, probably due to the acidity of the complex, since the medium was poorly buffered. However, the calcium-ligno-protein

complex had a marked favorable effect, especially in the absence of added inorganic nitrogen. Whether this is due to the fact that the microörganisms decomposing the glucose could utilize the complex as a source of nitrogen, or whether it is due to the favorable effect of the complex upon the development of the bacteria, especially the nitrogen-fixing organisms, remains to be determined.

The following experiment deals with the influence of ligno-protein complexes, as compared with that of lignin, on the one hand, and of soil humus (α-humus), on the other, upon the decomposition of cellulose by microörganisms. This experiment will tend to establish whether the synthesized humus complexes have (*a*) an injurious effect upon an important microbiological process, or a

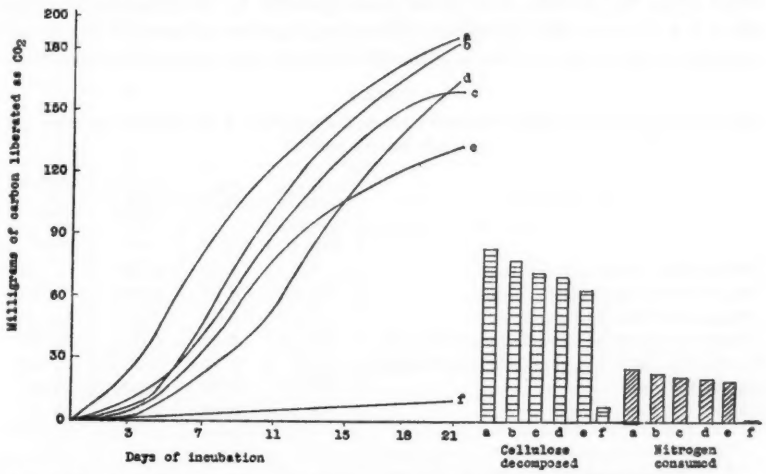

FIG. 1. THE INFLUENCE OF LIGNIN, LIGNO-PROTEIN COMPLEXES, AND SOIL "HUMUS" UPON THE DECOMPOSITION OF CELLULOSE BY MICROÖRGANISMS IN SAND MEDIA, AS MEASURED BY EVOLUTION OF CO_2

a, Ca-ligno-proteinate; b, alkali lignin; c, H-ligno-proteinate; d, soil "humus"; e, cellulose alone; f, cellulose without inorganic nitrogen.

favorable effect, or no effect at all, and (*b*) whether the nitrogen of the protein in the complex can be used as a source of nitrogen by the cellulose-decomposing organisms.

The experiment was carried out in a sand medium. In each of several long-necked flasks 100 gm. of washed sand and 1 gm. of ground air-dry cellulose were placed; the different humus preparations were then added and well mixed with the sand and the cellulose. To each flask 20 cc. of distilled water containing the necessary minerals was added. Some of the flasks received 200 mgm. of $NaNO_3$ and others received no inorganic nitrogen. The flasks were inoculated with a soil infusion and incubated for 21 days. The cultures were

aerated daily by passing a current of air above the medium, and the CO_2 was measured. At the end of the incubation period, the residual cellulose in the cultures was determined. In those cultures, which received additional inorganic nitrogen in the form of sodium nitrate, the residual nitrate was also determined. The results are reported in table 2 and in figure 1.

The results show that the ligno-protein complexes, as well as the soil humus (α-humus), cannot be used by the cellulose-decomposing bacteria as sources of nitrogen. Only traces of cellulose were decomposed in the absence of inorganic nitrogen, and in the presence of the organic nitrogenous complexes; the natural and synthesized humus complexes behaved practically alike. However, these complexes, as well as the lignin itself, have a decidedly beneficial effect upon the process of cellulose decomposition by microörganisms; this effect is not due to the utilization of these compounds as a source of carbon or nitrogen, as shown by the fact that the cellulose-nitrogen ratio was not modified,

TABLE 3

Influence of lignin and of ligno-proteinates upon the decomposition of Trichoderma mycelium and casein by microörganisms

MATERIAL ADDED	TOTAL CO_2 LIBERATED	NH_3 LIBERATED	α-HUMUS FOUND IN MEDIUM	NITROGEN IN α-HUMUS
	mgm. C	*mgm. N*	*gm.*	*mgm.*
Sand medium alone	1.7	0.56	0.003	....
Fungus mycelium, 1 gm.	105.2	6.02	0.047	4.62
Fungus mycelium + 2 gm. lignin	78.9	2.38	1.554	19.18
Fungus mycelium + 1 gm. H-ligno-proteinate	81.6	4.34	0.771	24.22*
Fungus mycelium + 1 gm. Ca-ligno-proteinate	119.7	7.70	0.706	23.66†
Casein, 0.5 gm.	105.5	53.20	0.006	2.80
Casein + 2.5 gm. lignin	74.7	38.08	2.000	18.48

* Original nitrogen in complex = 22.6 gm.

† Original nitrogen in complex = 22.8 gm.

but is due to some other factor. The Ca-ligno-proteinate exerted the most favorable action. Whether this is due to the colloidal properties of the organic complexes, to their buffering properties, or to some other phenomenon still remains to be determined. The interesting point to note in this connection is that the synthesized H-ligno-proteinate had exactly the same effect upon cellulose decomposition as the "humus" or "humic acid" of the soil, which is also largely a hydrogen complex.

It is of considerable interest to determine the influence of lignin and of the artificial humus complexes upon the decomposition of organic nitrogenous compounds, not only in the state of free protein but also in the form of complex substances, of a nature similar to those that one would expect to find in the soil. For this purpose, the mass of growth of the fungus *Trichoderma*, grown on synthetic liquid media, and comprising both mycelium and spores, was employed. This material was selected for two reasons: (a) this organism is a common, cellulose-decomposing soil fungus, and it was found to synthesize

considerable cell substance in the soil; (b) this cell substance has a fairly high protein content (6.4 per cent nitrogen) and one would expect that a part of its nitrogen would be liberated as ammonia in the process of its decomposition. The same medium was employed as in the previous experiment, except that 1-gm. portions of fungous growth, instead of the cellulose, were used with no additional inorganic nitrogen. At the end of the incubation period (15 days), the ammonia nitrogen was determined in an aliquot portion of the residue, while the remaining portion was extracted with 4 per cent NaOH solution; the extract was acidified with HCl; the precipitate was filtered, washed, dried

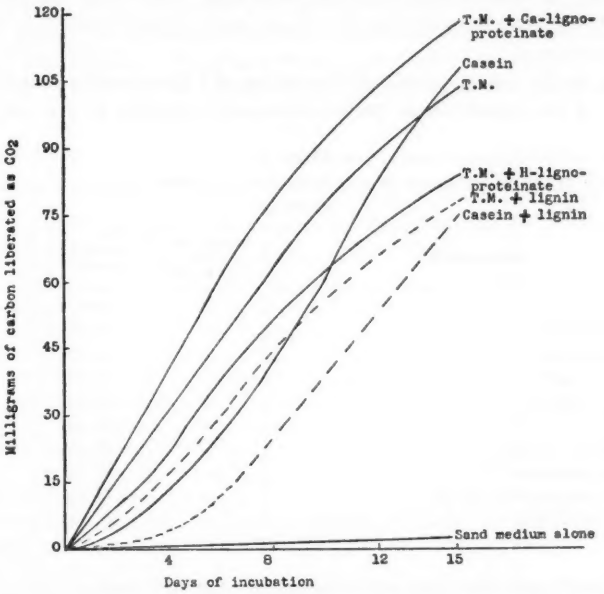

FIG. 2. INFLUENCE OF LIGNIN AND LIGNO-PROTEINS UPON THE DECOMPOSITION OF TRICHODERMA MYCELIUM (T. M.) AND CASEIN IN SAND MEDIUM, AS MEASURED BY THE EVOLUTION OF CO_2

weighed, and analyzed for total nitrogen. The results are given in table 3 and in figure 2.

Lignin alone repressed the decomposition of the fungous mycelium, as shown by the reduction in the evolution of CO_2. The previous experiment would lead us to conclude that this reduction is due to a depression in the decomposition of the proteins and not of the carbohydrates. That this was actually the case is proved by the reduction in the ammonia liberated. The ratio between the reduction in the carbon liberated as CO_2 (105.2 − 78.9 = 26.3) and the reduction in the nitrogen liberated as ammonia (6.02 − 2.38 = 3.64), was found to be 7.2. The ratio of the carbon to nitrogen in the mycelium is $\frac{50}{6.4}$ =

7.8. A similar depressing effect of the lignin was observed in the case of the casein. Here the ratio of C/N reduction is 2.0. The ratio of C/N in casein itself is about 3.0. The addition of the ligno-protein complexes produced a somewhat depressing effect in the case of the acid complex and a favorable effect for the calcium complex, with a corresponding liberation of the nitrogen as ammonia. In other words, the two ligno-protein complexes which are saturated with protein brought about no tying up of the protein. This is confirmed by the nitrogen content of the α-humus fraction of the residual material. Whereas the lignin tied up nearly 14.5 mgm. of nitrogen as protein from the *Trichoderma* mycelium and somewhat more from the casein, the ligno-protein complexes did not tie up any more protein than they have already contained.

These results not only prove the formation of a ligno-protein complex in the process of decomposition of protein-containing material in the soil, in the

TABLE 4

Influence of lignin and humus preparations upon the fixation of nitrogen by Azotobacter vinelandii

SUBSTANCES USED	INOCULATED WITH AZOTOBACTER	NITROGEN CONTENT	NITROGEN FIXED
		mgm.	*mgm.*
Control medium	−	0.35	....
Control medium	+	4.27	3.92
Lignin + $CaCO_3$	−	1.20	....
Lignin + $CaCO_3$	+	4.56	3.36
α-fraction + $CaCO_3$	−	9.80	....
α-fraction + $CaCO_3$	+	12.46	2.66
Ca-ligno-proteinate	−	10.22	....
H-ligno-proteinate + $CaCO_3$	+	15.62	5.40
Ca-ligno-proteinate	+	15.54	5.32

presence of lignin, but they also demonstrate that the tendency is toward the establishment of a complex between lignin and protein of a definite chemical composition. Once such a complex is formed, there will be no further protein absorption or removal of the protein from active decomposition by microorganisms. These phenomena have a highly important bearing upon the rapidity and amount of liberation of nitrogen in an available form, in the decomposition of plant and animal residues in the soil.

In the following experiments, the influence of the humus complexes synthesized in the laboratory, as compared with that of lignin and α-humus from soil, upon the fixation of nitrogen by *Azotobacter vinelandii* was determined. In a series of flasks were placed 100-cc. portions of 1.5 per cent mannitol solution, containing the necessary minerals, but no combined nitrogen, and adjusted to pH 8.0. One-half gram portions of the several preparations were added to the various flasks. These were then sterilized for 15 minutes at 15

pounds pressure, inoculated with a pure culture of *Azotobacter vinelandii*, and incubated for 21 days. The results (table 4) show that the lignin and the α-fraction of the soil had, under these experimental conditions, a somewhat depressive effect upon the fixation of nitrogen by the organism, probably due to their acid reaction, whereas the lignо-protein complexes had a decidedly beneficial effect.

In order to determine the nature of the stimulating effect of the synthesized humus complexes upon the fixation of nitrogen by Azotobacter, the last experiment was repeated under somewhat different conditions. In a series of 500-cc. flasks were placed 150-cc. portions of Ashby's medium. These flasks received varying quantities of calcium and iron ligno-proteinates, as well as of soil "humus" and of lignin. It has been shown by various investigators (3) that the addition of humus to pure cultures of Azotobacter increases considerably the

TABLE 5

Influence of lignin, ligno-proteinates, and "soil humus" upon nitrogen-fixation by Azotobacter vinelandii

TREATMENT	INOCULATED WITH AZOTOBACTER	NITROGEN CONTENT OF CULTURE	NITROGEN FIXED
		mgm.	*mgm.*
Control medium	−	0.50	....
Control medium	+	4.20	3.70
Lignin, 500 mgm.	−	1.20	....
Lignin, 500 mgm.	+	3.5	2.30
Ca-ligno-proteinate, 500 mgm.	−	9.80	....
Ca-ligno-proteinate, 50 mgm.	+	4.95	3.50
Ca-ligno-proteinate, 500 mgm.	+	13.20	3.40
Fe-ligno-proteinate, 500 mgm.	−	13.90	....
Fe-ligno-proteinate, 50 mgm.	+	7.70	5.85
Fe-ligno-proteinate, 500 mgm.	+	19.45	5.55
α-humus, 500 mgm.	−	15.40	....
α-humus, 500 mgm.	+	15.60	0.20

nitrogen-fixing capacity of this organism. Humus obtained from various sources was found to vary in its effect; humus prepared from sugars by boiling with acids has no such effect; on boiling humus with acid, the activating substance seems to be removed. The humus is not used by the organism either as a source of carbon or nitrogen. Burk et al. (1) have recently established the fact that the favorable effect of the humus upon nitrogen-fixation is due to its iron content.

The results presented in table 5 show that iron-ligno-proteinate had a decidedly favorable effect upon the fixation of nitrogen by the organism, but the Ca complexes, as well as the "natural soil humus," had no such effect. This "soil humus" was prepared by extracting soil with 4 per cent NaOH solution, acidifying the extract, boiling, filtering, washing, and drying; this "humus" had practically no favorable effect upon the growth of the organism above the

control and reduced considerably the fixation of nitrogen. Without attempting to analyze the nature of the action of the humus complexes upon nitrogen fixation by Azotobacter, it is sufficient to say that synthesized humus complexes, provided they contain iron, have an exactly similar favorable effect upon the process of fixation as has been reported for natural humus preparations which contain iron.

Samples of these synthetic preparations were submitted to Dr. D. Burk and his associates in the U. S. Department of Agriculture Bureau of Chemistry and Soils. The influence of these preparations was tested by them upon the growth of Azotobacter and Rhizobium, as determined by the Warburg technique, by measuring respiration, and by the relative turbidity; the synthetic preparations were compared with the action of natural humus preparations. The iron-ligno-proteinate was reported to compare quite favorably with "natural humic acids" containing iron, with respect to both induction period and amount of growth stimulation ultimately produced, and interestingly enough, in iron content.

DISCUSSION

The results presented in this paper contribute further information to the similarity in behavior between the "humus complexes" synthesized in the laboratory from lignin and protein and natural "humus complexes" isolated from soil and from peat. These results also show that in the process of decomposition of microbial cell substance, some of the protein constituents interact with the lignin to give rise to humus complexes. This tends to throw further light upon the rôle of soil microörganisms in synthesizing new complexes which contribute to the soil humus, either directly as the constituents of their cell substance, or indirectly, namely, by the interaction of some of the constituents of the microbial cell substance with some of the resistent constituents of the plant residues, especially the lignins.

SUMMARY

1. Results are reported on the influence of synthesized humus, or the humus-nucleus, upon certain important microbiological processes.

2. The synthesized humus complexes were found to have a favorable effect upon the decomposition of glucose by a mixed soil microbial population, especially in the absence of an added source of combined nitrogen.

3. Synthesized humus complexes as well as "natural soil humus" could not be used as sources of nitrogen by cellulose-decomposing microörganisms; however, they had a decidedly beneficial effect upon the decomposition of cellulose, in the presence of available combined nitrogen. The ratio between the cellulose decomposed and the nitrogen consumed by the microörganisms decomposing the cellulose was not modified by the presence of humus complexes.

4. Lignin was found to have no injurious effect upon cellulose decomposition; the effect was rather beneficial. This adds further weight to the idea

that the injurious effect of lignin upon protein decomposition is not due to any direct injury, but is indirect, namely, due to the formation with the protein of a complex, which is resistant to further decomposition by microörganisms, and which contributes to the formation of soil humus.

5. The decomposition of fungous mycelium was studied, in the absence and in the presence of lignin and ligno-protein complexes. Lignin was found to have an injurious effect upon this process, as measured by CO_2 liberation and NH_3 accumulation; a large part of the nitrogen of the mycelium could be isolated, at the end of the experiment in the form of a ligno-protein complex. The same was true of the influence of lignin upon the decomposition of casein.

6. Calcium-ligno-proteinate has a favorable effect upon the decomposition of the fungous mycelium, as measured by both CO_2 evolution and ammonia accumulation. The hydrogen-ligno-proteinate, however, had a slightly injurious effect, possibly due to its acid reaction.

7. The synthesized humus complexes containing iron had a highly beneficial effect upon the fixation of nitrogen by Azotobacter.

REFERENCES

(1) Burk, D., Lineweaver, H., Horner, C. K., and Allison, F. E. 1931 The relation between iron, humic acid and organic matter in the nutrition and stimulation of plant growth. *Science* 74: 522–524.

(2) Kaserer, H. 1912 Über die biologische Reizwirkung natürlicher Humusstoffe. *Centbl. Bakt.* (II) 31: 577–578.

(3) Krzemieniewski, S. 1908 Untersuchungen über Azotobacter chroococcum Beij. *Bul. Acad. Sci. Cracovie. Cl. Sci. Math. Nat.* 8: 929–1051.

(4) Olsen, C. 1930 On the influence of humus substances on the growth of green plants in water cultures. *Compt. Rend. Lab. Carlsberg* 18: 1–16.

(5) Remy, Th., and Rösing, G. 1911–1912 Über die biologische Reizwirkung natürlicher Humusstoffe. *Centbl. Bakt.* (II) 30: 349–384; 33: 618–623.

(6) Robertson, R. A., Irvine, J. C., and Dodson, M. E. 1907 A contribution to the chemistry and physiological action of the humic acids. *Biochem. Jour.* 2: 458–480.

(7) Söhngen, N. L. 1913 Einflusz von Kolloiden auf mikrobiologische Prozesse. *Centbl. Bakt.* (II) 38: 621–647.

BOOK REVIEW

Sulphur Bacteria. A Monograph. By DAVID ELLIS, Professor of Bacteriology, The Royal Technical College, Glasgow. Longmans, Green and Co., London, New York, 1932. Pp. IX + 261, figs. 66. Price, $7.50.

This is the first book in English to be added to the numerous treatises of these interesting autotrophic microörganisms. It is composed of 13 chapters, which are devoted principally to the following subjects: sources of hydrogen sulfide; metabolism, culture, classification, and description of the sulfur bacteria; influence of light on the organisms; the nature of the pigments of the organisms.

The author's objective, to develop " . . . . the present position of our knowledge of the sulphur bacteria," seems to have been incompletely attained. It is unfortunate that the book appeared at such a time as to miss the observations of van Niel (*Arch. Mikrobiol.*, 3 (1931): 1–112) which elucidate numerous previously confused features of the morphology and biochemistry of the purple and green sulfur bacteria.

Although little can be claimed for originality or completeness in the work, much that is interesting and informative can be derived by the discerning reader. However, certain defects appear which greatly detract from the value of the volume. These defects appear prominently in the biochemical discussions and start with the opening sentence: "The term Sulphur Bacteria is usually applied to the members of the group which have sulphur globules in their cells." This idea is retained throughout the work (*see* p. 84, 130, 220, 226) and the author excludes from the category of sulfur bacteria, those which are small rod-shaped forms generally classified among the Eubacteriales. Incidentally, this eliminates the sulfur bacteria which have received the most complete biochemical study at the present time. He appears to be unaware of the fact that the sulfur bacteria are not significant as physiological entities because of the presence of sulfur within the cells, but because of their ability to utilize the energy liberated in the oxidation of sulfur or incompletely oxidized compounds of sulfur. The products of oxidation are incidental and differ with the various organisms. It has further been shown by van Niel that storage of sulfur globules within the cells is dependent entirely upon the size of cells and has no other taxonomic, morphological, or biochemical significance.

His interpretation of the transfer of energy in the nutrition of the organisms is very confused and inconsistent with known facts, to judge only by the following typical examples: " . . . . this substance (H_2S) is not the source of either the food or the energy of the sulphur bacteria" (p. 47) and " . . . .

the change from sulphide to sulphur does not result in a liberation of energy" (p. 46). The impression is also given that the sulfur bacteria are unable to utilize the energy derived from oxidation of sulfur to sulfate (p. 33, 38).

Many misstatements are rather apparent. The following may suffice to illustrate the point. "The sulphate may be reduced to elementary sulphur if the supply of oxygen be scanty" (p. 8). (No direct microbiological change of this nature is known to the reviewer.) "No experimental evidence for the existence of this ferment (Philothion) has been supplied" (p. 24). (See however the glutathione of Hopkins and associates.) ". . . . the organism (*Thiobacillus denitrificans*) is one of the so-called obligate anaerobes" (p. 49). (This organism also develops aerobically.) "The sulphur compounds must be regarded as fermentable substances" (p. 50). (This is an unwarranted use of the term "fermentable substances.") Regarding *Thiobacillus thiooxidans*, he states, "The culture medium is acidified by its development, and the amount of thiosulfate increases steadily with the growth of this species." (p. 223). (The term "thiosulfate" is wrong and should be "sulfate.") "All of the chromoparus sulphur bacteria are purple" (p. 232). (There are also green forms.)

Morphology and classification occupy prominent portions of the volume. Although such information may be of aid in further studies, its value is limited in view of the great pleomorphism noted in those species which have received detailed study in the past. Without justification, the author appropriates the generic name *Thiobacillus* for one of the groups of the higher sulfur bacteria, although its use has been well established for a group of the sulfur bacteria included among the Eubacteriales, which the author naively would dismiss from the sulfur bacteria.

It is to be regretted that this group of organisms which is so fascinating from the biochemical viewpoint and which plays rôles of considerable economic importance in nature, should not have received more accurate and detailed consideration from the biochemical standpoint.

ROBERT L. STARKEY.